Ecology, Life History and Conservation Status of *Westralunio carteri* IREDALE 1934, an Endemic Freshwater Mussel of South-western Australia

Michael Willard Klunzinger

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Murdoch UNIVERSITY

DECLARATION

I declare that this thesis is my own original account of my research and contains as its main content, work which has not previously been submitted for a degree at any tertiary education institution and as author, I reserve the right of copyright under the *Copyright Act 1968*, Commonwealth of Australia.

MICHAEL W. KLUNZINGER

Frontispiece: Lindsay Marshall

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Abstract

Westralunio carteri, the only hyriid in south-western Australia, was nominated 'Vulnerable' (IUCN) in 1994. The aims of this study were to update the species' range and determine factors limiting its distribution, quantify tolerance to threats, quantify reproduction, describe glochidia morphology, identify host fishes to support the species' life cycle and estimate growth and age.

Extent of Occurrence (EOO) of *W. carteri* is currently 16,011.9 km^2, a 63.3% decline from the historic EOO of 43,579.8 km^2, suggesting that the species should be classified as 'Endangered' under IUCN guidelines. Multivariate analysis identified flow and drying as explaining most of the variation in the distribution data, while the difference between historic and current distribution was explained principally by salinity. Salinity tolerance experiments indicated LC_{50} values of 1.3 - 3.0 and LC_{95} of 3.2 - 4.3 g L^{-1}. Artificial water removal suggested *W. carteri* is intolerant of drying for more than five days during summer without shade or moist sediments.

Westralunio carteri spawns during winter; embryos are brooded in the gills of females to become glochidia and released on mucus strings in September – December, when they attach to fins of fishes. Glochidia morphology (size and larval teeth) is distinctive in *W. carteri*, compared to other Australian hyriids.

Glochidia were found on fins of seven native and three alien fish species from 18 populations. Prevalence was 0.0 - 41.0% and 9.2 - 90.5% and intensity 1.0 - 6.0 and 2.3 - 7.1 in alien and native fishes, respectively. Four native and one alien fish species were confirmed as competent hosts in the laboratory. Time to metamorphosis was 21-27 days.

Growth rates were ~12.0 to <0.1 mm yr^{-1} in the smallest (<30 mm long) and largest (>75 mm long) sizes. Calcein validated growth rings as annuli and ages were 3 – 51 years at shell lengths of 12.6 - 82.5 mm, respectively, from five populations. Growth rates and ages-at-length were highly variable between populations.

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Chapter 1

Review of Literature

1.1 Taxonomic diversity and biogeography of the Unionoida

The Bivalvia are an ancient class of molluscs with shells hinged into two parts. There are currently an estimated 9,200 extant species of bivalves comprised of 106 families, a majority of which inhabit marine and brackish waters (Bieler *et al*. 2010; Huber 2010). The subclass Palaeoheterodonta is composed of the Trigonoida, an order of fossil bivalves thought to have been extinct until the discovery of *Neotrigonia margaritacea* (LAMARCK, 1804) off the coast of Australia in 1802, and the Unionoida (Graf & Cummings 2006, 2007; Bieler *et al*. 2010). The Trigonoida are thought to be the sister group from which the Unionoida evolved as they colonised coastal rivers, migrating inland to adapt to a freshwater lifestyle (Graf & Cummings 2006, 2007; Bieler *et al*. 2010).

Freshwater mussels ('Unionoida' in Bauer & Wächtler 2001 and Strayer 2008; 'Unioniformes' in Bogan & Roe 2008; 'Unionida' in Bieler *et al*. 2010) are an ancient group of bivalve molluscs found on every continent except Antarctica. They live in freshwater lakes, rivers and wetlands (Bauer & Wächtler 2001). Globally, there are estimated to be between 796 (Bogan & Roe 2008) to 854 species (Graf & Cummings 2006, 2007) organised into two superfamilies: Unionoidea and Etherioidea, which are distinguished by larval form (Bauer & Wächtler 2001). The larvae of Unionoidea (Hyriidae, Margaritiferidae, Unionidae) are known as 'glochidia' and the larvae of the Etherioidea (Etheriidae, Iridinidae, Mycetopodidae) are either 'lasidia' or 'haustoria' (Bauer & Wächtler 2001), although Graf & Cummings (2006, 2007) placed the glochidia of the Hyriidae in the Etherioidea based on phylogenetic hypotheses. The distribution and number of species within each unionoid family (Graf & Cummings 2006, 2007; Bogan & Roe 2008) is presented in Table 1.1.

Table 1.1 Diversity and distribution of the Unionoida. (Graf & Cummings 2006, 2007; Bogan & Roe 2008).

Family	No. genera	No. species	Distribution
Etheriidae	4	4	South America; Africa; Madagascar; India
Hyriidae	16	76	Australasia; South America
Iridinidae	6	43	Tropical Africa
Margaritiferidae	1	13	Patchy distributions of North America; Western Europe; the Middle East; SE Asia; Japan; eastern Russia; NE China
Mycetopodidae	11	45	Central & South America
Unionidae	126	673	Widespread throughout North America; Eurasia; northern Middle East; NE Africa; southern Africa
Total	**164**	**854**	

The Australasian region of the Southern Hemisphere is dominated by the Hyriidae, with 32 (±5) species (Fig. 1.1) and South America, with 34 species (Graf & Cummings 2006, 2007; Bogan & Roe 2008). The Australasian region is represented by nine genera and 32 species of Hyriidae (McMichael & Hiscock 1958; Ponder & Bayer 2004; Walker 2004). Eighteen of the 32 species are found in Australia, represented by six genera (McMichael & Hiscock 1958; Ponder & Bayer 2004; Walker 2004; Table 1.2). New Guinea also contains two representatives from the Unionidae: Rectidentinae: *Haasodonta fannyae* (R.I. JOHNSON, 1948) and *Haasodonta vanheurni* MCMICHAEL & HISCOCK, 1958. Localised distributions of most species are generally patchy with densities typically ranging from 10 to 100 individuals $m^{2\ -1}$ (Bauer & Wächtler 2001; Strayer 2008), but one species has been known to reach densities of up to 814 $m^{2\ -1}$ (Ogilvie & Mitchell 1995).

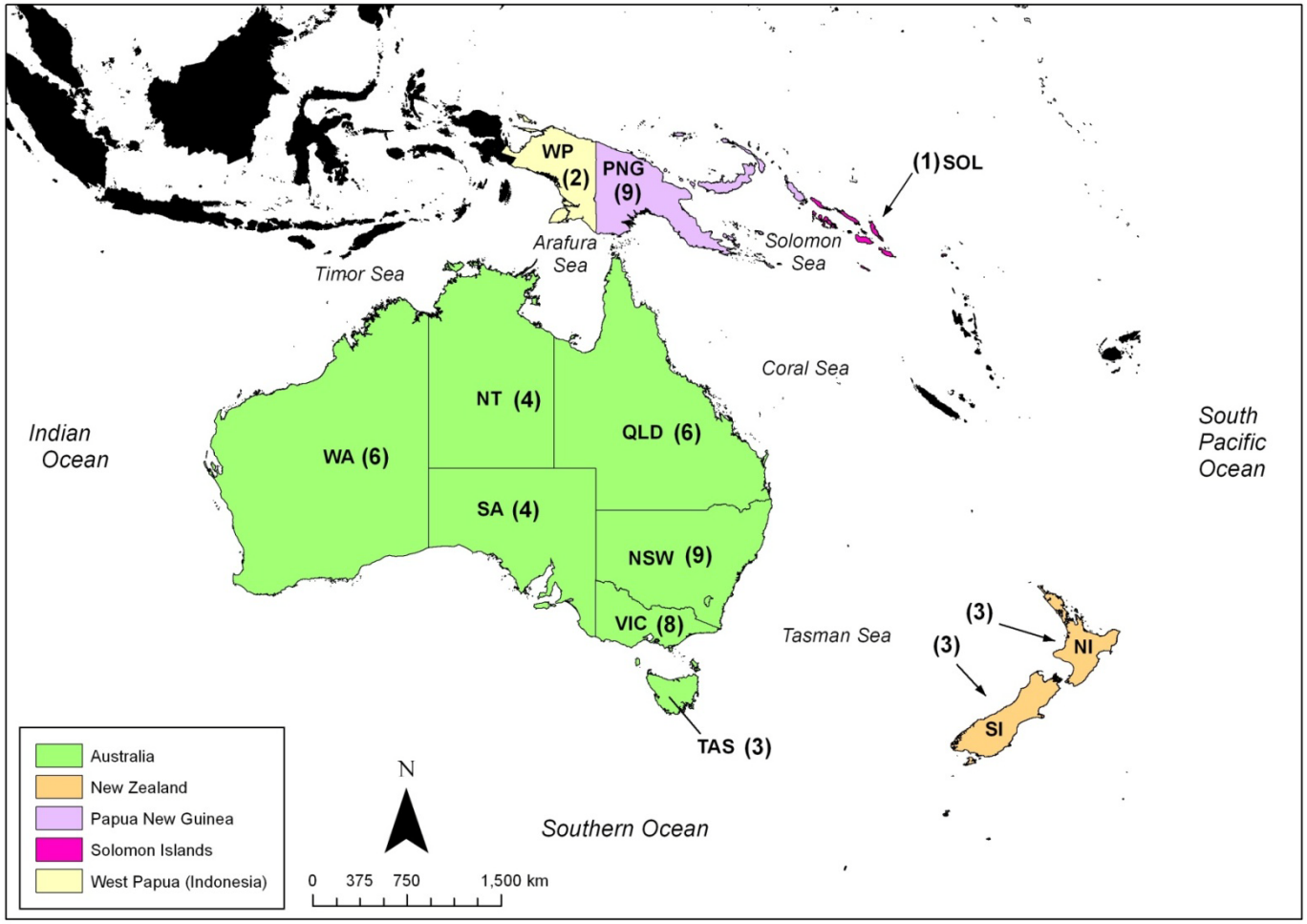

Fig. 1.1 The countries and territories of Australasia which contain 32 (± 5) species of Hyriidae. NSW = New South Wales; NT = Northern Territory; QLD = Queensland; SA = South Australia; TAS = Tasmania; VIC = Victoria; WA = Western Australia; NI = North Island and SI = South Island (New Zealand); PNG = Papua New Guinea; SOL = Solomon Islands; WP = West Papua (Indonesia). The number of species of Hyriidae in each locality is given in brackets.

Table 1.2. Diversity and distribution of the Australasian Hyriidae (McMichael & Hiscock 1958; Ponder & Bayer 2004; Walker 2004; Fenwick & Marshall 2006, unpubl.).
-Australia: MDB = Murray-Darling Basin; NSW = New South Wales, NT = Northern Territory, SA = South Australia, TAS = Tasmania, VIC = Victoria, WA = Western Australia, QLD = Queensland;
-PNG = Papua New Guinea;
-NZ = New Zealand (NI = North Island, SI = South Island);
-SOL = Solomon Islands;
-WP = West Papua (Indonesia).

Hyriidae: Hyriinae: Hyridellini	
Cucumerunio novaehollandiae (GRAY, 1834)	E. coast NSW, QLD
Hyridella australis (LAMARCK, 1819)	S.E. coast NSW, VIC
Hyridella depressa (LAMARCK, 1819)	S.E. coast NSW, VIC
Hyridella drapeta (IREDALE, 1934)	S.E. coast NSW, SA, VIC
Hyridella glenelgensis (DENNANT, 1898)	S.W. VIC
Hyridella narracanensis (COTTON & GABRIEL, 1932)	S.E. SA to E. VIC & N. TAS
Hyridella misoolensis (SCHEPMAN, 1897)	PNG
Hyridella guppyi (E.A. SMITH, 1885)	PNG, SOL
†*Cucumerunio websteri* (SIMPSON, 1902)	NI
†*Echyridella menziesii* (GRAY, 1843)	NI, SI
†*Echyridella lucasi* (SUTER, 1905)	SI
Echyridella onekaka FENWICK & MARSHALL, 2006	SI
Echyridella aucklandica (GRAY, 1843)	NI

Hyriidae: Velesunioninae	
Alathyria condola IREDALE, 1943	inland NSW, VIC + MDB
Alathyria jacksoni IREDALE, 1934	inland NSW, SA, QLD + MDB
Alathyria pertexta IREDALE, 1934	E. coast +Lake Eyre NSW, QLD. VIC
Alathyria profuga (GOULD, 1851)	E. NSW
Lortiella froggatti IREDALE, 1934	N.W. WA to N.W. NT
Lortiella rugata (G.B. SOWERBY II, 1868)	N.W. WA to N.E. NT
Lortiella opertanea PONDER & BAYER, 2004	Kimberly + Pilbara (WA), NT
Velesunio ambiguus (PHILIPPI, 1847)	E. coast & inland NSW, QLD, SA, TAS, VIC
Velesunio angasi (G.B. SOWERBY II, 1867)	N. WA to N. QLD
Velesunio moretonicus(REEVE, 1865)	N. TAS AU
§*Velesunio wilsonii* (LEA, 1859)	inland WA, NT & QLD + PNG
Westralunio carteri IREDALE, 1934	S.W. WA
Microdontia anodontaeformis (TAPPARONE CANEFRI, 1883)	PNG + WP
Velesunio ovata (HAAS, 1910)	PNG
Velesunio sentaniensis (HAAS, 1924)	PNG + WP
Virgus beccarianus (TAPPERONE CANEFRI, 1883)	PNG
Westralunio albertisi (CLENCH, 1957)	PNG
Westralunio flyensis (TAPPERONE CANEFRI, 1883)	PNG

§ Includes at least three undescribed species in central Australia (Baker *et al*. 2003, 2004). One specimen only recorded from New Guinea (McMichael & Hiscock 1958: p. 399).
†Recent work (M. Fenwick, B. Marshall, unpubl.) suggests these three species (two genera) should be synonymised as *Echyridella aucklandica.*

1.2 Biology of freshwater mussels

1.2.1 Life history stages

Fertilisation

Most freshwater mussels are dioecious, although some are hermaphroditic (van der Schalie 1945; Kat 1983). Males release sperm into the water column, which is taken in by females (Bauer & Wächtler 2001). Some species have been shown to release spermatozoa with heads embedded in spherical masses ('spermatozeugmata') and tails extended around the spheres; these are thought to facilitate sperm survival and transport when released into the water column (Ishibashi *et al.* 2000). Fertilisation occurs in the water tubes of the female's demibranchs in the gills (Bauer & Wächtler 2001). Although fertilisation occurs within the animal, it is considered to be external since the water tubes are part of the mantle cavity rather than the gonaduct (Fox 2005). Embryonic development takes place within modified spaces (marsupia) in the female's gills (Bauer & Wächtler 2001; Strayer 2008).

Population densities of mussels may affect dispersal of gametes and, therefore, influence fertilisation success (Strayer 2008). For example, some researchers suggest that fertilisation success of female *Elliptio complanata* (LIGHTFOOT, 1786) from a Quebec lake is a function of local population density (Downing *et al.* 1993). Similarly, increased aggregation (Burla *et al.* 1974; Amyot & Downing 1998) of sexually mature mussels or male-female coupling (Shelton 1997) during the spawning season implies that sperm dispersal and concentrations may be a limiting factor in female reproductive success.

On the contrary, other authors fail to report any evidence to suggest that successful fertilisation is a function of local population density (Young & Williams 1984; Fukuhara & Nagata 1995; Neves 1997; Haag & Staton 2003). Bauer (1991) and Walker *et al.* (2001) suggest that the ability of mussels living in low population

concentrations to develop into hermaphrodites would tend to increase fertilisation success at low population densities.

Larval stage

Embryos of Unionoida develop into larvae which are generally obligate parasites of fishes and this parasitic stage is thought to be the primary mechanism of population dispersal (Wächtler *et al.* 2001). Glochidia were first described as a species of parasitic bivalve, *Glochidium parasiticum* (RATHKE 1797) and later revealed as the larval stage of freshwater mussels (Carus 1832; Leydig 1866; Heard 2001; Heard & Dinesen 2001). Glochidia are brooded in the marsupia of the adult females' gills and develop into the juvenile freshwater mussel form while attached to their host through a metamorphosis of internal viscera and changes in shell development (Wächtler *et al.* 2001; Strayer 2008). Formation of the marsupia occurs in one or both pairs of gills (Mackie 1984): the outer or both pairs in Unionidae, the inner pair in Hyriidae, and both pairs in Margaritiferidae (Walker *et al.* 2001). Glochidia are held in the marsupia for a few weeks (tachytictic breeders) or up to several months (bradytictic breeders), depending on the species (Heard 1998).

Glochidia release occurs simultaneously from most individuals in a population within a few days or gradually over a longer time (Wächtler *et al.* 2001). Some species have been known to spawn more than once per year, such as *Cumberlandia monodonta* SAY, 1829 with two spawning periods (Gordon & Smith 1990), three spawning periods in *Velesunio ambiguus* PHILIPPI, 1847 (Walker 1981; Jones *et al.* 1986) or continuously throughout the year in *Velesunio angasi* SOWERBY, 1867 (Humphrey 1984). Depending on the size of glochidia and the size of the gravid adult mussel, fecundity ranges from several thousand to several million (Wächtler *et al.* 2001).

Glochidia morphology varies with shell shape, being triangular, spherical or hatchet-shaped with maximum length ranging from 47 to 400 μm (Lefevre & Curtis 1910; Jones *et al.* 1986; Wächtler *et al.* 2001). The edges of the glochidia shell are often equipped with hooks, known as 'larval teeth' which vary in morphology (Wächtler *et al.* 2001). In *Margaritifera* spp., larval teeth are very small or absent (Panha 1995; Pekkarinen & Englund 1996; Araujo & Ramos 1998). Larval teeth are also absent in *Diplodon iheringi* SIMPSON, 1900 (Bonetto 1959, 1961a; Dreher-Mansur 1998; Pimpão *et al.* 2012). Toothed glochidia generally attach to both gills and body surfaces of their hosts whereas the toothless varieties are generally restricted to gills (Wächtler *et al.* 2001). Through comparative observations, the smaller and toothless glochidia are the more specialized type with restricted distribution, whereas the larger glochidia with larval teeth are generally less specialised and more widely distributed (Wächtler *et al.* 2001).

Once released from the marsupia, glochidia have a brief chance to contact a host and survival is reported to last from two to 14 days unless they can attach to a host (Mackie 1984). Under laboratory conditions, if glochidia are kept in suspension more than three days, the number of surviving glochidia decreases to a very small fraction (Walker 1981; Sylvester *et al.* 1984). In some species, glochidia release is motivated by the presence of fish (Zale & Neves 1982a). For the majority of species, the high number of glochidia released increases the chance that at least some will contact a host (Wächtler *et al.* 2001).

Glochidia that are ready to attach to host fish perform snapping movements using a well-developed adductor muscle that closes their hinged shells (Walker 1981). This behaviour can be stimulated by adding sodium chloride crystals or fish mucus to glochidia suspended in solution (Lefevre & Curtis 1910; Hoggarth & Gaunt 1988; Walker *et al.* 2001). Sensory hairs, located in the soft body tissue of glochidia, are

thought to respond to chemical and mechanical stimuli, triggering the adductor muscle to contract (Wood 1974). Once glochidia attach to host fish, the adductor muscle remains contracted and is thought to degenerate during metamorphosis on the fish (Wächtler *et al.* 2001).

Freshwater mussels use a variety of strategies to attach glochidia onto hosts. Some use modified mantles as fishing lures to attract hosts (Kraemer 1970; Haag & Warren 1997; Haag *et al.* 1999; Corey *et al.* 2006). Some package glochidia in conglutinates that resemble prey items of predatory fishes (Jones *et al.* 1986; Haag *et al.* 1995; Hartfield & Hartfield 1996; Haag & Warren 2003). Some catch and hold fish and pump glochidia into their gills (Barnhart 2006), although it should be noted that some species bypass the parasitic stage altogether (Barfield & Watters 1998; Lellis & King 1998; Corey 2003).

Following attachment to host fish, the fish epithelial cells migrate to enclose the glochidia in a cyst and if glochidia survive immune response from the fish, they remain protected and may receive nutrients from the body fluids of their host (Arey 1932a, b, c; Rogers-Lowery & Dimock 2006). An epithelium formed by large cells with apical microvilli line the inner surface of glochidia and is thought to function in nutrient uptake from fish tissue after cyst formation (Herbers 1914; Arey 1932a, c; Pekkarinen & Englund 1996). Spherical particles from the apex of larval mantle cells, transported by lateral cilia bands appear to be storage granules for late parasitic stages (Scharsack 1994).

While attached to their host fish, glochidia undergo a reorganisation of viscera to prepare the parasitic larvae for a life as a young filter feeder (the juvenile stage). The key events during metamorphosis are: 1) the degeneration of the single larval adductor muscle and subsequent formation of a pair of definitive adductor muscles; 2) the formation of mantle tissue for food uptake and shell secretion; 3) the formation of a pair

of digestive glands; 4) formation of nerves; 5) growth of a large ciliated foot; and 6) primary stages of gill formation; metamorphosis is accompanied by an increase in DNA, RNA and protein synthesis (Wächtler *et al.* 2001; Fisher & Dimock, Jr. 2002a,b). The duration of the larval period lasts from three days (Seshaiya 1969) to several months (e.g. Coker *et al.* 1921; Young & Williams 1983a; Watters & O'Dee 1999) and as long as 10 months in *Margaritifera margaritifera* LINNAEUS, 1758 (Bauer 1987c).

Juvenile stage

Very little information is available about the juvenile stage, but most are thought to live in sediments (Yeager & Saylor 1995; Sparks & Strayer 1998; Smith *et al.* 2000) and some species produce a byssal thread (e.g. Brusca & Brusca 1990). Once metamorphosis of glochidia is complete, the newly formed juvenile mussel is ready to release from the fish (Bauer & Wächtler 2001; Strayer 2008). Release from the host fish begins by opening the epithelial cyst, with the exception of *Mutela bourguignati* MARTENS, 1897, which detaches from the distal end of the haustorium (Wächtler *et al.* 2001). Generally, juveniles at this stage are about the same size as glochidia, with glochidia of *M. margaritifera* and lasidium of *M. bourguignati* as exceptions, growing considerably during the larval stage from 50 to 450 µm and 200 µm to 1.5 mm, respectively (Wächtler *et al.* 2001).

The characteristic shell structure of juveniles includes an elastic hinge as well as anterior and posterior adductor muscles which close the two valves (Bauer & Wächtler 2001; Strayer 2008). Shell growth and growth rings depend on the species and the growth temperature preferred (Bauer & Wächtler 2001; Strayer 2008). Some species have shell pores which may function by improving gas exchange (Bauer & Wächtler 2001; Strayer 2008). Shell formation takes place on the interior ciliated epithelium of the mantle, which replaces the glochidia mantle that forms after encystment (Bauer &

Wächtler 2001; Strayer 2008). In most cases, benthic life begins before the gills are completely developed. Juveniles are far more mobile than their adult counterparts, due to the elongated foot which is capable of rapid peristaltic movement (Wächtler 1986; Yeager *et al.* 1994). This becomes very useful when young mussels drop off the fish in unfavourable habitat sites and move to a more favourable place (Wächtler 1986).

The digestive system of juveniles consists of a ciliated mouth opening surrounded by cirrae, a digestive gland with diverticula and a stomach followed by an uncoiled intestine; in largely transparent juveniles, the onset of food intake is apparent by yellow or green content of the digestive gland (Maaß 1987; Niemeyer 1992). Active post-glochidial food intake occurs via water flow caused by cilia at various sites (Strayer 2008). The gill papillae contribute, but the primary structure involved in particle stream flow is the foot with a ciliary groove (Strayer 2008). Presumably, with increasing differentiation and development of the gills, the adult manner of feeding is gradually adopted by juveniles (Wächtler 1986). The juveniles may also deposit feed, supplementing suspension feeding (Yeager *et al.* 1994).

The preferred habitat of early post-parasitic juveniles is within or above the upper few centimetres of sediment which is well-aerated (Young & Williams 1983b; Neves & Widlak 1988; Buddensiek 1991; Buddensiek *et al.* 1993). In this area, juveniles are particularly vulnerable to sediment-bound toxic material (Salomons *et al.* 1987; Yeager *et al.* 1994). The juvenile stage is thought to last from one to several years (Coker *et al.* 1921; Jirka & Neves 1992; Haag & Staton 2003).

Adulthood

Having reached sexual maturity, most adults are more or less epifaunal, living on or near the sediment surface (Bauer & Wächtler 2001; Smith *et al.* 2000; Strayer 2008), although Schwalb & Pusch (2007) report infaunal species, living within sediments.

Freshwater mussels live for one to several decades (e.g. Bauer 1992; Haag & Staton 2003; Howard & Cuffey 2003). The long life span of Unionoida may be an adaptation to deal with highly variable environments, similar to the way plants use seed dormancy to deal with temporal variability in rainfall (Levin 1992). Some authors suggest that most estimates of unionoid life spans are flawed by a factor of three to five (Strayer 2008). Specific details of life history (e.g. seasonal timing, fecundity, size of larvae, age at sexual maturity and maximum life span) vary across and within species (Bauer 1994; Watters & O'Dee 1999; Bauer & Wächtler 2001; Haag & Staton 2003).

1.2.2 Growth and longevity

Lines or rings that form on the outer surface of the shell are thought to indicate periods when growth is seasonal, e.g. interrupted during cooler winter periods, in periods of low water (stress), or from some physical disturbance or other undesirable condition (Ghent *et al.* 1978; Parmalee & Bogan 1998). Some researchers however, suggest that specimens had patterns that were not supportive of this hypothesis (Negus 1966; Haukoja & Hakala 1978; Downing & Downing 1992; Downing *et al.* 1992). A precise detailed mechanism of periostracum and shell formation is uncertain, but some models suggest that growth rings appearing on the outer shell surface are the result of continued periostracum (the outer horn-like shell protein) formation building loops on top of itself during a time when formation of the internal calcareous layers cease (Petit *et al.* 1978, 1979, 1980a, b; Saleuddin & Petit 1983; de Paula & Silveira 2009).

The technique of cross-sectioning freshwater mussels to observe growth interruption lines within the internal shell layers as a reflection of changes in factors that affect growth is established (Clark 1980; Jones 1980; Neves & Moyer 1988; Schöne *et al.* 2004; Valdovinos & Pederos 2007; Haag & Commens-Carson 2008). A number of researchers recommend independent validation of these growth rings and recent work

has established that the examination of growth rings from internal calcareous layers is a valid method for estimating age by cross-referencing with growth in freshwater mussels (Haag & Commens-Carson 2008; Rypel *et al.* 2008; Haag 2009).

Walker (1981) and Humphrey (1984) have investigated growth and shell ring formation in Australian hyriids, but Walker *et al.* (2001) point out that although growth lines in shell cross-sections that may be annual, growth may also be arrested by flood and drought and should be validated for each population.

1.3 Factors affecting distribution and abundance of freshwater mussels

1.3.1 Dispersal

The function of dispersal is to allow species to move into previously unoccupied areas and expand their geographic range, and connect subpopulations within established ranges to maintain metapopulations (Strayer 2008). Some range boundaries are set by climate (e.g. Clarke 1973); however, some evidence suggests that barriers to dispersal can limit unionoid range boundaries, which often end at drainage divides, despite similar ecological conditions on the other side of the divide (Ortmann 1913; van der Schalie 1938, 1945; van der Schalie & van der Schalie 1963). Linking drainage divides by canals and stocking of host fish from one divide to another can also influence dispersal (e.g. Strayer & Jirka 1997; Clayton *et al*. 2001).

Dispersal also connects isolated populations, increasing genetic diversity within the metapopulation (Strayer 2008). Some species are highly variable genetically within and across drainages (e.g. Nagel 2000; Hughes *et al.* 2004; Mock *et al.* 2004; Geist & Kuehn 2005; Geist *et al.* 2006), suggesting low dispersal rates, while other species show less differentiation (Elderkin *et al.* 2007) inferring a higher dispersal rate.

Being obligate parasites of fishes, glochidia attachment and metamorphosis to the juvenile stage on the host has been suggested to be an important factor in population

dispersal (Bauer & Wächtler 2001; Strayer 2008). Actual dispersal rates vary widely across species, in part because dispersal varies so widely among host fishes (Rodriguez 2002). Small benthic fish are often frequent hosts of unionids (Cummings & Watters 2005). These fishes, however, have very limited mobility; suggesting that dispersal rates of unionids that depend on these fishes will be low over great distances (McLain & Ross 2005; Petty & Grossman 2004). Freshwater mussels which use highly mobile or migratory hosts, however, may have a greater rate of dispersal throughout their range (Strayer 2008).

1.3.2 Habitat

Various species of freshwater mussels require different habitats (Ortmann 1919; Coker *et al.* 1921; Baker 1928; van der Schalie 1938; Clarke 1981; Neves & Widlak 1987; Layzer & Madison 1995; Strayer & Jirka 1997; Parmalee & Bogan 1998; Hastie *et al.* 2000; Strayer 2008). Nevertheless, habitat requirements of freshwater mussels are often vague and unsatisfactory for three reasons (Strayer 2008): 1) quantitative tests of association between mussel distributions and factors such as sediment grain size, current speed, water depth, and distance to shore are generally ineffective at predicting occurrence or abundance (Strayer 1981, 1999; Holland-Bartels 1990; Strayer & Ralley 1993; Strayer *et al.* 1994; Balfour & Smock 1995; Vaughn & Pyron 1995; Johnson & Brown 2000; Brim-Box *et al.* 2002; Gangloff & Feminella 2007); 2) habitat descriptions are not transferable, i.e. mussels living in a well-defined habitat in one site may often occur at very different habitats at other sites (Coker *et al.* 1921; Strayer 1981), possibly because the actual controlling factor is not the presumed controlling variable, but some other unmeasured factor (Strayer 2008); 3) even when habitat descriptions successfully describe distribution across a range of sites, they may give little inference to actual controlling factors (Strayer 2008).

An alternative to traditional approaches in determining mussel habitat usage is to look at what the mussel requires from its habitat, rather than a list of observations when a stream is visited during low flow periods (Strayer 2008). The primary requirements for establishing and maintaining a freshwater mussel population include juvenile settlement, sediment stability, water current speed, dissolved oxygen and calcium delivery, food delivery (see Section 1.3.4 below), water temperature, mussel density and habitat quality (Strayer 2008).

Juvenile settlement and sediment support

Suitable mussel habitat must be stable enough to establish juveniles without being swept away by currents (Layzer & Madison 1995; Payne & Miller 2000; Hardison & Layzer 2001; Myers-Kinzie *et al.* 2002). Excessively soft and excessively compact sediments are likely to limit the spatial distribution of mussel populations. There have been few attempts to test the theory that sediments must be firm but penetrable to suit freshwater mussels. Johnson & Brown (2000) did, however, show that *Margaritifera hembeli* CONRAD, 1938 occurred more often in compact sediments than in very soft ones, which was quantified using a penetrometer, suggesting sediment compaction is an agent for sediment stability. Lake-dwelling unionids are generally absent from very soft sediments in deep water (Headlee 1906; Ghent *et al.* 1978) although other factors, such as cold hypolimnetic temperatures, low dissolved oxygen, and low food concentrations may also be responsible for their absence at great depths (Cvancara 1972).

Freshwater mussels move slowly, yet occupy one of the most unstable habitats worldwide (Leopold *et al.* 1964; Gordon *et al.* 1992; Strayer 2008). Thus, mussels may only occur in patches of the stream bed that are particularly stable (Vannote & Minshall 1982; Young & Williams 1983a; Layzer & Madison 1995; Strayer 1999; Johnson & Brown 2000; Hastie *et al.* 2001; Howard & Cuffey 2003; Morales *et al.* 2006; Gangloff

& Feminella 2007), some of which may be protected from floods (Miller & Payne 1998; Strayer 1999; Hastie *et al.* 2001).

Areas that are stable during floods must also be submerged at low flows and drought to be suitable habitat to support mussels (Layzer *et al.* 1993; Strayer 2008). Areas close to shore that are exposed at low stream flows are also unsuitable habitats for mussels (Miller & Payne 1998; Gagnon *et al.* 2004; Golladay *et al.* 2004). Sudden changes in sediment or water flows can induce lateral or vertical instability in stream channels (Leopold *et al.* 1964; Brookes 1996).

Habitat structure may provide protection from predators (Strayer 2008). Juveniles buried in sediments may be shielded from epibenthic predators, such as fish and predatory crustaceans (Strayer 2008). If such habitat is missing or rendered unsuitable, juveniles may become vulnerable to increased predation (Sparks & Strayer 1998).

Water current speed

Water current speed can affect mussel filtration rates (Aldridge *et al.* 1987) and create food-depleted boundary layers above mussel beds, which may affect food intake, growth and reproduction (Bolden & Brown 2002); an observation especially notable in marine bivalves (Wildish & Kristmanson 1997) and freshwater dreissenids (Karatayev *et al.* 2006), but little research has been done on freshwater mussel populations (Strayer 2008).

Dissolved oxygen and calcium delivery

The provision of dissolved oxygen (DO) for respiration and calcium for shell growth are important factors provided by habitats to sustain freshwater mussel populations (Strayer 2008). Adult mussels may have some tolerance to hypoxia or anoxia for several weeks,

by closing their valves and undergoing anaerobic respiration (Sheldon & Walker 1989; McMahon & Bogan 2001). Prolonged exposure to anoxic conditions can, however, cause reductions in growth or cause females to abort their glochidia (Aldridge & McIvor 2003) and if anoxia persists, mussels can die (Golladay *et al.* 2004).

Juveniles are much less tolerant of anoxic conditions than their adult counterparts (Dimock & Wright 1993; Sparks & Strayer 1998; Dimock 2000). Dissolved oxygen within the sediment habitat where juveniles live is up to ~90% lower than overlying water (Buddensiek *et al.* 1993; Strayer *et al.* 1997). When organic matter, fine sediments, and sewage pollution decreases interstitial DO, juvenile populations can suffer (e.g. Hynes 1960).

Although mussels' shells are composed of calcium carbonate ($CaCO_3$), they can survive at very low concentrations of dissolved calcium due to their ability to efficiently utilise calcium absorption from water and ingesta as well as shells which are resistant to dissolution (McMahon & Bogan 2001). Some mussel species manage to survive where ambient calcium concentrations can be less than 5 mg L^{-1} (Rooke & Mackie 1984; Huebner *et al.* 1990), although in calcium concentrations of less than 1 mg L^{-1}, freshwater mussel populations may be limited (Forsyth 1978; Timperley 1987).

Temperature

High temperatures are thought to be responsible for mussel deaths in droughts (Gagnon *et al.* 2004; Golladay *et al.* 2004), have a negative impact on glochidia viability (Zimmerman & Neves 2002) and increase mortality from other stressors such as copper (Jacobson *et al.* 1997). At low temperatures, growth is much slower; Beaty & Neves (2004) for example, showed that juvenile *Villosa iris* (LEA, 1829), a North American unionid, grew at a rate of 0.809 μm day^{-1} $°C^{-1}$ above 15°C and predicted growth was 16 μm day^{-1} at 20°C.

On the same principal, freshwater mussel growth may also decrease at very high temperatures with an increase in respiration rates (e.g. Pandolfo *et al*. 2010). Huebner (1982), for example, showed that in populations of *Lampsilis radiata* (GMELIN, 1791) sampled from Manitoba, Canada mean Q_{10} values were 3.3 when grown in a temperature range of 6.5°C to 17.5°C and 3.4 at 17.5°C to 23.5°C. Similarly, Myers-Kinzie (1998) found that Q_{10} was 3.4 when mussels were grown in the 15-25°C temperature range and mean respiration rates ranged from 0.149 mg O_2/L/g body weight/hr at 10°C to 0.909 mg O_2/L/g body weight/hr at 25°C. Schöne *et al.* (2004) suggested that growth was highest during summer months in Swedish *M. margaritifera* when 217-year growth rates were reconstructed from shells. Hastie *et al.* (2003) showed that high temperatures increased juvenile recruitment of *M. margaritifera* in the United Kingdom and that glochidia growth rates increased with increasing temperatures while on host fish. Others observed that mussel growth rates (in the Northern Hemisphere) were greater in the south than in the north, where temperatures were higher and growing seasons were longer (Bauer 1992; Chamberlain 1931). Bauer (1992) also noted that such higher growth rates were correlated with shorter life spans.

In general, higher temperatures speed up development (e.g. Dudgeon & Morton 1984; Van Snik *et al.* 2002; Hastie & Young 2003; Hastie *et al.* 2003; Steingraeber *et al.* 2007). Temperature is important to the timing of life history events (Hastie & Young 2003) matching habitat suitability conditions (e.g. flow rates for settlement) with availability of migratory fish hosts. Such events coincide with temperature changes and seasonality. Very low temperatures have been found to completely halt reproduction; freshwater mussels found downstream from hypolimnetic-release dams failed to reproduce following release of water from the dams (Heinricher & Layzer 1999). Others (Roberts & Barnhart 1999) found that glochidial transformation was most

successful at low temperatures, perhaps because low temperatures suppress fish immune function (Strayer 2008).

1.3.3 Hosts

Some evidence suggests that a few freshwater mussel species do not necessarily need to parasitise host fish (Bonetto 1961b; Barfield & Watters 1998; Lellis & King 1998; Corey 2003) and some use amphibians as hosts (Barnhart *et al.* 1998; Watters & O'Dee 1998). The number of known host fish species varies across mussel species, but most mussel species have from two to 20 known hosts (Cummings & Watters 2005). A few mussel species are highly specialised, having only one host species (Knudsen & Hove 1997; Lee & Hove 1998; Baird 2000), while others are highly generalised in selecting fish hosts, such as *Strophitus undulatus* (SAY, 1817) with 36 known host species from seven families (van Snik *et al*. 2002).

Although glochidial prevalence rates in mixed-species fish populations are often less than 10% (Weir 1977; Neves & Widlak 1988; Weiss & Layzer 1995), when comparing glochidial prevalence within known hosts, peak prevalence rates are often high (50-100%) (see Tedla & Fernando 1969; Dartnall & Walkery 1979; Trdan 1981; Zale & Neves 1982a, b; Young & Williams 1984; Bauer 1987a; Cunjak & McGladdery 1991; Jokela *et al.* 1991; Weaver *et al.* 1991; Riusech & Barnhart 2000; Hastie & Young 2001; McLain & Ross 2005).

Mussel species that have large glochidia may transform in a shorter time on fish and can, therefore, evade the immune systems of more fish species (Bauer 1994). Host specialist species could be less common and more vulnerable to human impacts than host generalists; as suggested by a database of North American glochidia-host species relationships in which host generalists appear to be widespread and common, whereas threatened species are more likely to be host specialists (Cummings & Watters 2005).

Individual host fishes may vary in their importance in supporting freshwater mussel recruitment, as suggested by (O'Brien & Brim-Box 1999) who found glochidia metamorphosis success varied across different host species in the laboratory. Patterns of host fish utilisation are non-random, with some groups of fish with glochidia prevalence higher than others (Jeschke & Strayer 2005). For example, host fishes more closely associated with benthic mussel habitat are more likely to encounter glochidia, whereas open water, cold water and cave-dwelling species may be less likely to encounter glochidia (Strayer 2008). The behaviour, seasonal migrations, local distributions, and abundance of various host species are also important and can influence the actual exposure of each host to mussel glochidia and its effectiveness as a competent host (Martel & Lauzon-Guay 2005).

Some studies indicate that there may be key intraspecific variation in host relationships, where mussels are more capable of using fish populations with which they co-occur than fish of the same species from other basins (Bauer 1987b; Rogers *et al.* 2001; Wächtler *et al.* 2001). Within a known host, both incursion rates and the number of glochidia per infected host (intensity) in nature often are lower on old, larger fish than smaller, younger fish (Tedla & Fernando 1969; Young & Williams 1984; Bauer 1987a; Hastie & Young 2001), which is consistent with laboratory findings; meaning that older fish may develop glochidial resistance from previous infections. This correlation is not always the case (Jokela *et al.* 1991; Blažek & Gelnar 2006) and the degree to which immune response and resistance is responsible for this relationship is unclear.

Once attached to the host fish, glochidia mortality is generally high (>50%) (Hastie & Young 2001; Jansen *et al.* 2001) and rejection from the incorrect host generally occurs within hours or days after cyst formation (Scharsack 1994). Mortality could be density-dependent, although this is unclear (Bauer 1987b). After repeated

exposure, host fish can develop absolute immunity and acquired immunity to glochidia infections (Reuling 1919; Arey 1921, 1923, 1932b). Therefore, the success of glochidia to metamorphose to the juvenile on an individual fish decreases with persistent infestations (Rogers & Dimock 2003; Dodd *et al.* 2005). It may take several infestations for host immunity to develop fully (Rogers & Dimock 2003; Dodd *et al.* 2005) and eventually fades with age (Dodd *et al.* 2006). Glochidia survival on resistant fish ranges from 26 to 100% of the survival on immunologically naïve fish (Rogers & Dimock 2003; Dodd *et al.* 2005. Also, transformation success in laboratory experiments seems to be lower on older and larger fish than on young, smaller fish, even if none of these fish were exposed to glochidia (Bauer 1987a). In some cases, infected fish develop cross-resistance to glochidia of other, related mussel species (Dodd *et al.* 2005).

Immunity is important to consider when thinking about the factors that affect freshwater mussel ecology because: 1) immunity may cause intraspecific competition for hosts, inferring density-dependent response between mussels and host fish which might control mussel populations; and 2) cross-species immunity could infer interspecific competition for hosts (Strayer 2008). Kirk & Layzer (1997), for example, successfully induced development of glochidia to young mussels on several species of 'non-host' fish after treating the fish with cortisol as an immunosuppressant. Whether immunity is strong enough in nature to lead to significant host competition and whether this ultimately affects freshwater mussel ecology in uncertain (Strayer 2008).

Australian host fish diversity

Glochidia hosts are known for only eight of the 18 species of freshwater mussels in Australia, including *Alathyria jacksoni* IREDALE, 1934, *Hyridella australis* LAMARCK 1819, *Hyridella depressa* LAMARCK 1819, *Hyridella drapeta* IREDALE 1934, *Velesunio*

ambiguus PHILIPPI 1847, *Velesunio angasi* (SOWERBY 1867), *Velesunio moretonicus* (REEVE 1865) and *Westralunio carteri* IREDALE 1934 (from Hiscock 1951; Atkins 1979; Walker 1981; Humphrey 1984; Widarto 1993 and DPIPWE 2009; Klunzinger *et al*. 2012a). Although glochidia hosts for the other 10 species of Australian freshwater mussels are unknown, from those species which have been studied, Australian Hyriidae appear to be host generalists, using a variety of fish species as hosts (Walker *et al*. 2001; Klunzinger *et al*. 2012a).

1.3.4 Food

Unionoida are able to capture a wide range of particulate matter that may serve as food including large bacteria, phytoplankton, small zooplankton, organic detritus, and perhaps dissolved organic material (Vaughn & Hakenkamp 2001). Different species of freshwater mussels overlap in the types of particles they capture (Jorgensen *et al*. 1984; Bisbee 1984; Parker *et al*. 1998) and may possibly compete for food (DiDonato 1998). Captured food sources may or may not be digested, while others are probably essential to survival, growth, and reproduction (Vaughn & Hakenkamp 2001). Juvenile and adult mussels also feed off of sediment food sources through pedal and siphonal feeding (McMahon 1991; Gatenby *et al*. 1996). Nichols & Garling (2000) also suggest that bacteria ingestion may be especially important in carbon metabolism.

Food limitation

An individual becomes limited by food when growth, size, survival, fecundity, or fitness could be improved through increased quantity and quality of food (Edwards & Edwards 2011). As fecundity is a strong function of body size (Byrne 1998; Haag & Staton 2003), if well-fed mussels grow faster and larger, increasing and improving food resources to individuals may have a positive effect on population size. Evidence for

food limitation of a mussel population is best exemplified by the effects of the zebra mussel invasion on Unionoida in the Hudson River that started in 1992, causing phytoplankton and small zooplankton to drop by 80-90% (although suspended bacteria concentration increased) (Caraco *et al.* 1997; Findlay *et al.* 1998; Caraco *et al.* 2006). Simultaneously, unionid populations fell by 65-99.7% between 1992 and 1999. Drastic changes in these mussel populations were not attributed to zebra mussel bio-fouling, as had been observed elsewhere (Haag *et al.* 1993; Ricciardi *et al.* 1995; Schloesser *et al.* 1996), but more likely that zebra mussels severely reduced food resources for Unionoida, and food-limitation increased mortality and decreased recruitment (Strayer 2008).

Mussels are frequently abundant in areas of high phytoplankton biomass (Ostrovsky *et al.* 1993; Vaughn & Hakenkamp 2001), indicating that these populations may be food-limited. One study proposed that the somewhat high metabolic rates of Unionoida limit population size in food-poor habitats, whereas the slower-metabolizing *M. margaritifera* is able to cope, suggesting that species-specific differences in food-limitation may decide their distribution and abundance (Bauer 1991). In another study, researchers observed that unionoid growth rates were rapid in two Rhode Island ponds, but nearly zero in a nearby pond and that in mussels translocated from the fast-growing populations to the locality with slow growing mussels, growth decreased, probably as a result of food availability (Kesler *et al.* 2007). Similarly, an experimental translocation of the hyriid *H. depressa* from an oligotrophic lake to stream sites above and below a sewage treatment plant showed that mussels moved to enriched sites had greater growth and twice the fecundity of mussels in less productive sites (Byrne 1998; Walker *et al.* 2001).

In some cases, freshwater mussels are most likely to be food-limited as a result of their own feeding in lakes if 1) their population density is large, 2) the water column

is shallow, 3) phytoplankton growth rates are small, 4) the response of mussel feeding and increased phytoplankton growth is weak, and 5) the water column is poorly mixed (MacIsaac *et al.* 1999; Ackermann *et al.* 2001; Edwards *et al.* 2005). In flowing waters, the situation becomes more complicated as food can be transported from upstream, from the bottom, and from the river banks. These complexities limit the possibility of simple models predicting how often and under what circumstances Unionoida are likely to control particulate concentrations and structure (Kryger & Riisgård 1988). As in the lakes situation, the impacts mussels have on food resources are greatest with high mussel densities, shallow water columns, and low algal growth rates which occur in turbid, shaded, and nutrient poor waters (Strayer 2008).

Individual mussels and populations may be food-limited if environmental conditions negatively impact the mussels' ability to feed, regardless of the ambient food concentrations and although this research has not been performed with Unionoida, it has been addressed for other bivalves (Strayer 2008). In studies with zebra mussels, for example, a high ratio of inorganic to organic particles limits mussel growth (Madon *et al.* 1998; Schneider *et al.* 1998). Also, strong currents and high concentrations of suspended solids could inhibit bivalve feeding (Aldridge *et al*. 1987; Wildish & Kristmanson 1997). How frequently such environmental conditions cause food-limitation in freshwater mussels is uncertain (Strayer 2008).

1.3.5 Predators, parasitism & disease

Predators

Little is known about the geographic extent and impacts predators have on freshwater mussel populations, but predators of North American and European freshwater mussels include muskrats (Convey *et al*. 1989; Hanson *et al*. 1989; Neves & Odom 1989), raccoons (Gagnon *et al*. 2004; Golladay *et al*. 2004), river otters (Morejohn 1969;

Pennak 1978; Toweill 1974), skunks (Hazard 1982; Pennak 1978), large salamanders (Pennak 1978), fishes (Adams 1892; Baker 1916; Coker *et al.* 1921; Fuller 1974; McMahon 1991; Williams *et al*. 1993), turtles (Pennak 1978) and birds (Berrow 1991; van Tets 1994). Invertebrates including crayfish (Klocker & Strayer 1994), micro turbellarians (Coker *et al.* 1921; Delp 2002; Zimmerman *et al.* 2003), chaetogastrine oligochaetes, cyclopoid copepods, tanypodine chironomids and insects (Coker *et al.* 1921; Walker 1981; Strayer 2008) predate on juvenile Unionoida. Many studies have shown that muskrats eat a great number of Unionoida and are selective on size and species (Bovbjerg 1956; Convey *et al.* 1989; Hanson *et al.* 1989; Neves & Odom 1989; Watters 1994; Jokela & Mutikainen 1995; Tyrrell & Hornbach 1998; Diggins & Stewart 2000; Zahner-Meike & Hanson 2001). The effects of predators on limiting mussel populations are unknown (Strayer 2008).

In Australia, several animals including fish, turtles, water rats, birds, and crayfish are thought to actively hunt and feed on freshwater mussels (van Tets 1994; Walker *et al.* 2001). Freshwater mussels are also an important source of food for Indigenous people (van Tets 1994; Scott 2000). The extent and impact of these predators on hyriid populations in Australia does not appear to have been studied.

Parasitism and disease

Freshwater mussels harbour parasites which use the mussel as part of their life cycle and also may ingest other parasites which may not use freshwater mussels as hosts, but are rather like 'sinks' for anything living in the water column that is sucked in through the mussels' inhalant siphon or live as commensal organisms on the shell surfaces (e.g. Fuller 1974). Digenetic trematodes (Digenea), for example, which use mussels as an intermediate host, can castrate their hosts and prevent mussel reproduction (Jokela *et al.* 1993; Martell & Trdan 1994; Taskinen *et al.* 1994; Widarto 1993; Walker *et al.* 2001).

Aspidogastrid trematodes (Aspidogastrea) are also common in freshwater mussels (Coker *et al.* 1921; Huener 1984; Duobinis-Gray *et al.* 1991), but their effects are unknown. Ergasilid copepods are known parasites of mussels, but their effects are uncertain (Saarinen & Taskinen 2004; Taskinen & Saarinen 2006). Unionicola mites also parasitise freshwater mussels and are quite common, with prevalence rates often as high as 90% (Mitchell 1965; Edwards & Dimock 1988; Virdine & Wilson 1991), but their effects are unknown. Other species of shellfish are known to concentrate *Giardia* and *Cryptosporidium* parasites, which can cause severe diarrhoea when they infect animals and humans (Wolfe 1992; Fayer *et al.* 1997; Graczyk *et al.* 1999a, b, 2000; Lewis 2004).

Bitterlings (Cyprinidae: *Rhodeus* AGASSIZ, 1832 spp.) have a specialised reproductive strategy where the parent fish transfer responsibility for the care of their young to various species of freshwater mussels (Unionidae and Margaritiferidae). Females extend her ovipositor into the mantle cavity of the mussel to deposit eggs between the gill filaments, which is subsequently followed by males ejecting sperm into the mussel's inhalant siphon and fertilization takes place within the gills of the host unionoid (e.g. Reynolds *et al*. 1997; Aldridge 1999).

1.4 The utilitarian view of mussels in freshwater ecosystems

1.4.1 Importance in ecosystem function

Freshwater mussels in aquatic ecosystems are important as suspension-feeders, impacting water chemistry and clarity, and the amount and type of suspended particles in water (Welker & Walz 1998; Vaughn & Hakenkamp 2001; Strayer 2008). They act as natural boilers, connecting benthic and pelagic regions in freshwater lakes and rivers. Living mussels and their shells provide or improve habitats by giving physical structure, stabilising and mixing sediments, directly or indirectly controlling food availability for

other organisms through biodeposition of organic material and nutrient flux and providing micro-refugia for benthic organisms (Chatelain & Chabot 1983; Beckett *et al.* 1996; Vaughn *et al.* 2002; Gutierrez *et al.* 2003; Zimmerman & de Szalay 2007). Mussel waste enhances local concentrations of algae (Vaughn *et al.* 2007) and macroinvertebrates (Vaughn & Spooner 2006). Historically, freshwater mussels have had economic importance as a source of pearls, mother-of-pearl, and food for human consumption (e.g. Kunz 1898; Morrison 1942; Claassen 1994; Ziuganov *et al.* 1994; Anthony & Downing 2001; Walker *et al.* 2001).

1.4.2 Mussels as bioindicators of freshwater health

Mussels have been widely utilised as 'bioindicators' of aquatic contaminants in freshwater, marine and estuarine ecosystems and as a direct measure of metal bioavailability (Atkins 1981; Allison & Simpson 1989; Storey & Edward 1989; Chu *et al.* 1990; Berrow 1991; Zatta *et al.* 1992; Mersch & Johansson 1993; Camusso *et al.* 1994; Humphrey 1995; Naimo 1995; Nelson *et al.* 1995; Ryan *et al.* 2008). Mussels readily accumulate metals through the transport of water across their gills and through ingestion of benthic sediment particles. Because of their sedentary nature, longevity, wide distribution and tolerance of high trace metal concentrations, their metal accumulations can reflect contamination history of a certain environment (Phillips 1977; Naimo 1995; Jamil *et al.* 1999). Because of their ability to reflect contamination history, freshwater mussels have been labelled as 'good' indicators of biological health and water quality (Grabarkiewicz & Davis 2008).

1.5 Conservation of freshwater mussels

Freshwater ecosystems may be the most endangered ecosystems in the world (Dudgeon *et al.* 2005). Freshwater molluscs, including mussels, are particularly vulnerable,

having had more extinctions than that of mammals and birds combined (Ponder 1998; Seddon 1998). The plight of Unionoida is well described (Bauer 1988; Bogan 1993; Neves 1993; Williams *et al.* 1993). Humans have had major impacts on the factors that influence unionoid populations (i.e. dispersal, habitat, hosts, food, predators, parasites and disease), often resulting in population decline and even extinction (Strayer 2008). Some evidence suggests that the decline of freshwater molluscs may be a global phenomenon (Lydeard *et al*. 2008), but few quantitative reports on the subject exist, apart from North America (e.g. Neves 1997; USFWS 2007), Europe (e.g. Araujo & Ramos 2000; Seddon 2000; Young *et al.* 2001a, b) and Australia (e.g. Walker *et al.* 2001; Brainwood *et al.* 2006; Jones & Byrne 2010). Specific threatening processes that have caused the decline of Unionoida include river regulation, loss of suitable habitat, and loss of host fishes, anthropogenic pollution, invasive species and commercial exploitation (Walker *et al.* 2001; Lydeard *et al.* 2008; Strayer 2008).

River regulation

Modelling and empirical analyses indicate that artificial barriers, such as dams, could influence mussel metapopulations, and because of the longevity of some mussel species, the effects of the barriers may not yet have been observed (Watters 1996; McLaughlin *et al.* 2006; Strayer 2008). Worldwide, there are an estimated 45,000 large dams, which may be absolute barriers for mussel distribution, primarily by the impediment of host fish movements (Watters 1996; McLaughlin *et al*. 2006). There are also millions of small dams throughout the rivers and streams of the world which may have similar effects (Jackson *et al.* 2001; Malmqvist & Rundle 2002; Katopodis & Aadland 2006). Other barriers could include long stretches of stream habitats that have become unsuitable for fish or mussels (Strayer 2008). These barriers will encumber, to some degree, the ability of mussels to adjust their population ranges to changing climate

(Hastie *et al*. 2003) and coldwater releases from hypolimnetic dams can negatively impact breeding (Heinricher & Layzer 1999).

Loss of suitable habitat

Humans have also changed the physical and chemical components of lentic and lotic habitats through changing hydrology (Nilsson *et al.* 2005), sediments (Waters 1995) and nutrient loading (Carpenter *et al.* 1998). Temperature, light penetration or impedance, physical changes in shoreline structure, forest clearing, row cropping, urban development, filling of shallow water, construction or deconstruction, water diversion, riparian habitat destruction, in stream substrate mining and water pollution may have also caused widespread harm to mussel populations worldwide (Trimble 1981; Hartfield 1993; Waters 1995; Brookes 1996; Brim-Box & Mossa 1999; Doyle *et al.* 2003).

Loss of host fish

Many of the factors which have affected mussel populations directly have also affected their host fish (Trautman 1981). Humans have also spread fish species outside their natural ranges (Fuller *et al.* 1999; Rahel 2000; Allen *et al.* 2002; Morgan *et al.* 2004), spread infectious fish diseases across the globe (e.g. Bartholomew 2002; Marina *et al.* 2008), and over harvested freshwater fish stocks (Nepszy 1999; Allan *et al.* 2005). As a result, very few systems globally still maintain their original fish communities (e.g. Bianco 1995; Morgan *et al*. 1998; Allen *et al*. 2002; Allan *et al*. 2005). So, by changing the structure of fish communities, impeding fish movements, introducing feral fish, and foreign fish diseases, humans have changed the availability of host fishes with which endemic freshwater mussels would have traditionally been found (Strayer 2008).

Pollution

The extent to which toxins affect the distribution and abundance of mussel populations is not yet clear. Most studies of the effects of toxins on freshwater mussels have focussed on defining lethal concentrations (LC_{50}), but the sublethal effects of toxins on growth, reproduction, behaviour, interactions between toxins or between toxins and other controlling factors are largely unknown. Toxicological results have not often been classed demographically, except when the toxin is so lethal that it completely destroys the population (Strayer 2008). Freshwater mussels appear to have been eliminated from many areas by anthropogenic toxins, including acid mine drainage (Ortmann 1909; Neves *et al.* 1997), ammonia (Augspurger *et al.* 2003), chlorine and chlorine by-products (Goudreau *et al.* 1993), heavy metals (Naimo 1995), industrial chemical spills (Crossman & Cairns 1973; Sparks *et al.* 1999; USFWS 2002), synthetic pesticides (Conners & Black 2004), and polycyclic aromatic hydrocarbons (Weinstein & Polk 2001). The toxic compounds that are of particular concern to freshwater mussel populations are un-ionized ammonia (Newton 2003; Augspurger *et al*. 2003; Mummert *et al.* 2003), toxic materials with a high affinity for sediments (Naimo 1995), and endocrine disruptors (Goudreau *et al*. 1993).

Ammonia comes from the decomposition of organic matter and the reduction of nitrate to ammonia (DNRA) (e.g. Kelso *et al.* 1997; Burgin & Hamilton 2007) and is generally the predominant form of inorganic nitrogen in hypoxic or anoxic conditions (Wetzel 2001). Ammonia is more abundant in sediments than in overlying water and exists in two forms, the ammonium ion NH_4^+ and NH_3, un-ionized ammonia. The balance between these forms depends on pH and temperature (Emerson *et al.* 2007). Un-ionized ammonia (NH_3) is extremely toxic to at least juvenile freshwater mussels with a 96-hr lethal concentration (LC_{50}) of only 40-280 μgL^{-1} in the species which were tested (Augspurger *et al.* 2003; Mummert *et al.* 2003; Newton 2003). Increased

inorganic pollution, increased autochthonous generation of organic material from nutrient loading and increased inputs of silt and clay have all acted to increase interstitial ammonia concentrations where mussels live, and toxic effects are most severe when pH and temperatures are high, which leads to a high proportion of un-ionized ammonia (Vitousek 1994; Waters 1995; Vitousek *et al.* 1997; Brim-Box & Mossa 1999). This is likely to occur during summer low-flow periods in high temperature streams, which are highly alkaline, received high inputs of nitrogen and fine sediments and can result in freshwater mussel population declines (Howells *et al.* 1996; Poole & Downing 2004). High ammonia or low DO could be responsible for recruitment failures in *M. margaritifera* within streams that have very compact sediments clogged with fine particles (Geist & Auerswald 2007).

Many pollutants, such as heavy metals, organochlorine pesticides, polychlorinated biphenyls and polyaromatic hydrocarbons are not stable in water and sink into sediments, which are not easily washed from the mussels' habitats and may linger long after the source of contamination has been eliminated. Juveniles that live in the sediments are particularly vulnerable to these toxins (Naimo 1995).

Endocrine disruptors mimic natural hormones and can disrupt reproduction and physiology of freshwater mussels; these include human or agricultural pharmaceuticals, organochlorine pesticides, tributylin antifouling paints, and breakdown products of detergents which are now widespread in surface waters (Kolpin *et al.* 2002). These chemicals can interfere with normal reproduction in freshwater mussels or their host fishes; for example, endocrine disruptors in sewage effluent have caused sex change in freshwater mussels (Blaise *et al.* 2003) and induced spawning (Gagné *et al.* 2004).

Salinisation

Salinisation of freshwater is a global problem which significantly affects areas such as central and South America, south-western North America, the Middle East, central Asia, South Africa and parts of Australia (Williams 2001). Very few reports suggest that populations of freshwater mussels have declined from salinisation of their formerly freshwater habitats, although salinity has been implicated as a major threat to freshwater mussel populations in south-western North America (Lang 2001), northern Europe (Hastie *et al.* 2003), as well as south-eastern and south-western Australia (Kendrick 1976; Walker 1981; Williams *et al.* 1991).

Invasive species

Invasive bivalves, such as *Corbicula* spp. and dreissenids in North America can displace native freshwater mussel species by greatly depleting food sources and bio-fouling native species (Ricciardi *et al*. 1995). The Asian clam (*Corbicula fluminea* (O.F. MÜLLER, 1774)) and the golden mussel (*Limnoperna fortunei* (DUNKER, 1857)) invasion of South America have caused similar problems (Darrigran 2002).

Feral pigs are the most abundant free-ranging alien ungulate in North America (Sweeney *et al.* 2003), found mainly on floodplains along rivers; in addition to causing saltation by uprooting stream bank vegetation, they also directly consume freshwater mussels (SCDNR 2005). Feral pigs also cover much of Australia and cause similar problems (Choquenot *et al.* 1996).

Commercial exploitation

Humans have a history of exploiting mussels for their shells for various purposes. In China, for example, freshwater mussels have been cultured for pearl production for more than 2,000 years (Dan & Ruobo 2002). Exports of North American shell to the

Japanese pearl industry (Bowen *et al.* 1994), collection of Scottish and Irish *M. margaritifera* for the pearls they produce (Beasley *et al.* 1998), and exploitation of shells to make buttons in North America (MacWillie *et al.* 1914) and South America (Beasley 2001) have led to population declines. Freshwater mussels are exploited as a human food item, particularly in Asian markets; including Endangered species such as *Margaritifera laosensis* (LEE, 1863) (see Köhler *et al.* 2008).

1.6 Freshwater mussels of south-western Australia

The South West Coast Drainage Division is one of 12 drainage divisions which were defined by the Australian Water Resources Council in the 1960s (AWRC 1976; Fig. 1.2). The region is isolated by deserts and the Southern and Indian Oceans which has led to a greater degree of endemicity of aquatic macrofauna, including freshwater mussels (100%), freshwater crayfishes (100%) and freshwater fishes (82%), than any other drainage division in Australia (Morgan *et al.* 2011).

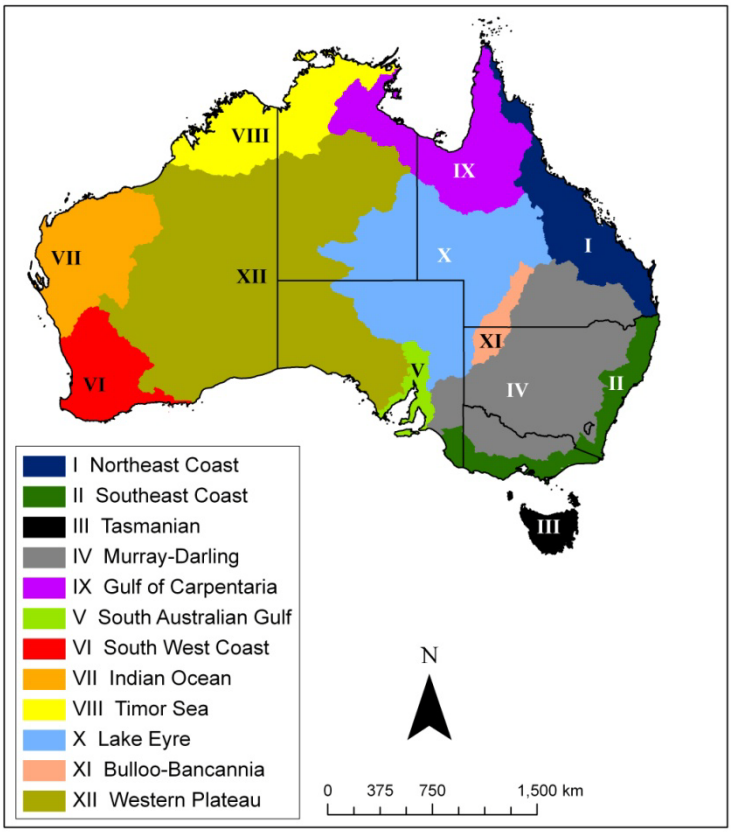

Fig. 1.2 Drainage Divisions of Australia. (AWRC 1976).

Carter's Freshwater Mussel *Westralunio carteri* IREDALE, 1934 is an Australian hyriid only found in south-western Australia, where it is the only species of Hyriidae and the only unionoid to inhabit the region (Iredale, 1934; McMichael & Hiscock 1958; Walker 2004). Furthermore, the species is the only member of the genus *Westralunio* to reside in Australia; the other two species (*W. albertisi* (CLENCH, 1957) and *W. flyensis* (TAPPERONE CANEFRI, 1883)) are found in Papua New Guinea (McMichael & Hiscock 1958; Walker *et al*. 2001; Walker 2004).

Kendrick (1976) first reported the species' demise when he found populations were disappearing from the Avon River, presumably as a result of salinisation of freshwater. A few studies have used the species as a bioindicator of river contaminants (Storey & Edward 1989; Bennet-Chambers *et al.* 1999). The species was nominated for addition to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, following a river health monitoring program in the late 1990s (Halse *et al*. 2002; Pinder *et al.* 2004), which resulted in its listing as Vulnerable (IUCN 1996). A taxon is Vulnerable when it is not Critically Endangered or Endangered but is facing a high risk of extinction in the wild in the medium-term future. The listing was changed in 2011 to 'Least Concern' (Köhler 2011). This species is listed as a Priority Fauna with a ranking of Priority 4 (P4) by the Department of Environment and Conservation, Government of Western Australia; a P4 species is defined as Rare, Near Threatened and other species in need of monitoring (DEC 2011). Despite its threatened status, little is known about the species' habitat preferences, tolerance to environmental stressors or its life cycle. Furthermore, details of the species' ecology are largely unknown and therefore the current study represents the first investigation to centre specifically on the ecology and biology of any species of freshwater mussel in Western Australia.

1.7 Aims and hypotheses

In this thesis, I determine the historical and contemporary range of *W. carteri* and elucidate the environmental variables that most influence its distribution, quantify its tolerance to variables associated with key environmental stressors, determine its reproductive development, describe its glochidium, identify its potential and definitive host fishes, quantify growth rates and validate annuli formation in shells to infer instantaneous ages-at-length. The following hypotheses are tested.

- **Chapter 2**: *Westralunio carteri* is assumed to have declined or disappeared from rivers which have been affected by secondary salinisation (IUCN 1996), yet salinity tolerance of the species has not been formally tested. I hypothesise that *W. carteri* will have a salinity tolerance similar to other Australian hyriids which have been tested (e.g. Walker 1981) and thus will not be found in systems which have salinities which are typically greater than 3 g L^{-1}.

  Given that a number of other factors are known to have caused declines in freshwater mussels elsewhere in Australia (e.g. Jones & Byrne 2010) and abroad (summarised in Strayer 2008), it is likely that *W. carteri* will either not be found or will have undergone or be undergoing localised extirpation or decline in areas impacted by physical, chemical or biological disturbance (e.g. sedimentation, low pH, eutrophication, trampling by livestock), particularly given that the region has been greatly affected by land use change since European colonisation. Furthermore, if they follow the same trend as Australian freshwater fishes (e.g. Morrongiello *et al.* 2011), populations of *W. carteri* are unlikely to be found in non-perennial or other systems affected by drying.

- **Chapter 3**: Given that other hyriids from temperate regions of Australia, which have climates similar to that of south-western Australia (Jones *et al*. 1986; Byrne 1998), I hypothesise that *Westralunio carteri* has a distinctly seasonal

reproductive period, which coincides with other aquatic fauna in the region including freshwater crayfishes (e.g. Beatty *et al*. 2003) and fishes (Morgan *et al*. 1998, 2011). Furthermore, glochidia release from *W. carteri* is likely to coincide with seasonal migrations of fishes, when potential hosts are highly likely to come into contact with gravid *W. carteri*.

- **Chapter 4**: Like other glochidia of the Velesunioninae (see Walker *et al*. 2001 and Walker 2004), glochidia of *W. carteri* are likely to have a pair of interlocking blade-like larval teeth on opposing valves, unlike their Hyriinae (Hyridellini) counterparts (see Jones *et al*. 1986; Jupiter & Byrne 1997) which have bifurcated larval teeth. The shell size and shape of *W. carteri* will be useful in identifying glochidia attached to host fishes.
- **Chapter 5**: Walker *et al*. (2001) suggested that the glochidia of Australian Hyriidae are host generalists, using more than one species and most native or endemic fishes to complete their life cycle, but introduced cyprinids are unlikely to be competent hosts. Thus, I expect that, like the other Australian hyriids which have been studied, *W. carteri* will have multiple native and endemic host fishes, but introduced (alien) cyprinids are unlikely to be competent hosts.
- **Chapter 6**: Growth and age estimates are largely unknown for the majority of Australasian hyriid species, but in a review by Walker *et al*. (2001), the authors suggest that Australian hyriids may live for at least 40 years and stress the importance of validating growth and age estimation in each test population because growing conditions may be variable in different systems. Given that winter growth interruption lines have been validated as true annuli in the otoliths of freshwater fishes of the region (e.g. Pen & Potter 1990, 1991a, b; Morgan *et al*. 1995, 2000, 2002); I expect the same will be true in the shells in *W. carteri*.

  Because non-annual 'pseudo-annuli' have been shown to occur from

handling (e.g. Downing *et al*. 1992 in Haag 2009), I hypothesise that calcein can be used as an *in situ* growth marker in adult *W. carteri*, which was established as an effective, non-invasive method to mark the shells of North American juvenile unionids (Eads & Layzer 2002) and other bivalves (van der Geest *et al*. 2011) for validating growth. I anticipate that growth ring counts will be a more accurate reflection of age than growth modelling in *W. carteri* as has been suggested for other species of freshwater mussels elsewhere (e.g. Haag & Rypel 2011).

I conclude with **Chapter 7** by summarising the study as a whole, determining whether these hypotheses were answered, provide a brief review of knowledge gaps and recommend future areas of study for *W. carteri*. The chapters are written as manuscripts, two of which (Chapters 4 and 5) have been published. Freshwater invertebrate conservation is a strong theme of this thesis, but the reader is advised that chapter subjects, while intrinsically important to the conservation of *W. carteri* may be written in a theme targeted for a specific journal. Also, I tread lightly with explicit conservation management recommendations given that there will still be much left to learn about *W. carteri* even after this thesis study is completed and because governments have legislative policies (the *Western Australian Wildlife Conservation Act 1950* and the *Commonwealth of Australia Environment Protection and Biodiversity Conservation Act 1999*) which declare that conservation listings must first undergo an assessment process and are generally followed with written conservation management plans and actions, usually in consultation with species' experts.

Chapter 2

Factors affecting the distribution and conservation status of *Westralunio carteri*

2.1 Introduction

The International Union for the Conservation of Nature (IUCN) Red List of threatened species is widely recognised as one of the most authoritative sources of information on the conservation status of plants and animals (Lamourex *et al.* 2003; Rodrigues *et al.* 2006). The utility of the Red List derives from a clear and objective set of criteria for determining and reviewing conservation status, based on population size, generation length, rate of decline, extent of occurrence (EOO) and area of occupancy (AOO), as well the publication of the data used to support the listing in a searchable, online format (IUCN 2011).

Although initially designed to evaluate and document extinction risk of individual species, the Red List is increasingly being used to inform conservation policies and legislation, prioritise areas for conservation action, guide the planning of conservation reserves and support environmental monitoring (Possingham *et al.* 2002; Miller *et al.* 2007; Szabo *et al.* 2012). The use of the IUCN Red List in conservation policy and planning places an increasing demand on high quality data for the correct application of listing criteria. In practice, however, such data are often lacking, especially for invertebrates (Newton 2010; Cardoso *et al.* 2011). The Red List guidelines explicitly state that "the absence of high quality data should not deter attempts at applying the ciriteria", but application of the criteria with inadequate data on the distribution and abundance of invertebrate taxa may lead to under-representation or misrepresentation of invertebrates in the Red List and the various indices derived from it (Cardoso *et al.* 2012).

In addition to categorising conservation status, the IUCN Red List may also detail threatening processes for each species (Cassini 2011). In order to define the extent of a species occurrence or area of occupancy, knowledge of limiting processes or threats is necessary to determine occupiable habitats and limits of the species range (Gaston 1991). However, there are currently no objective criteria for determining threat rankings. As a consequence, the listing of threatening processes lacks the rigour of the process for listing conservation status and often relies on expert opinion, with the potential for being compromised by local biases and lack of agreement between experts (Haywood 2009). Cassini (2011) promotes the use of species distribution models to generate objective rankings of threats for listed species. Species distribution models relate field observations of species presence/absence or abundance at known locations with information on the environmental or spatial characteristics of those locations (Elith & Leathwick 2009). They are typically used to explain the ecological or evolutionary drivers of species distributions and to predict changes in species distributions over space and time, making them suitable for ranking threats in a more objective fashion than is currently employed. Species distribution models, while extremely useful in identifying and ranking threats, are based on correlations between species occurrence/abundance and environmental predictors, so the causal inferences that can be drawn from them are constrained by covariation among environmental variables, which can only be separated through controlled experimental studies (Yuan 2007). Experimental confirmation of threats has been shown to verify field observations (e.g. Kefford *et al*. 2004; Beatty *et al*. 2011).

Of the 12666 species in the latest IUCN Red List of Threatened Species, ~6% are Extinct and ~94% are Vulnerable, Endangered or Critically Endangered (IUCN 2011). As a percentage of the number of threatened species within each taxon, molluscs have the

greatest extinction rate of any other taxonomic group and non-marine bivalves have a greater percentage of extinctions (27.8%) than any of the other threatened molluscan species (Ponder & Walker 2003; IUCN 2011). Freshwater mussels (Bivalvia: Unionoida) comprise 70.3% of world non-marine bivalve diversity (Bogan & Roe 2008; Huber 2010). Multiple factors are responsible for the decline of freshwater fauna and Unionoida are particularly sensitive due to their sessile nature and limited dispersal ability (Bauer & Wächtler 2001; Strayer 2008).

South West Western Australia is a recognised biodiversity hotspot (Myers *et al.* 2000). Although depauperate in terms of the number of species, the region has the greatest degree of freshwater mussel, fish and crayfish endemicity than anywhere else in Australia (Morgan *et al.* 2011). Contributing to this measure, *W. carteri* is the only freshwater mussel in the region and the only member of the genus in Australia (McMichael & Hiscock 1958; Walker 2004; Morgan *et al.* 2011). Following a biodiversity survey in the south-western Australian agricultural zone in 1995 (Pinder *et al.* 2004), *W. carteri* was nominated as 'Vulnerable' under criteria A1c, B1 + 2bc of the IUCN Red List (IUCN 1996), although there was no formal publication of the species' distribution and abundance. A recent re-assessment of the conservation status of *W. carteri* led to a change in status to 'Least Concern' (Köhler 2011). The justification used for the change in conservation status was that the species "...is widespread in Western Australia, is a habitat generalist, and is resistant to organic pollution", although again, these comments did not reference any detailed published information on the distribution and abundance of the species. In this chapter I provide, for the first time, an estimate of the current range of *W. carteri*, compare this with the range estimated from historical data, and use species distribution modelling to infer potential threatening processes for the species. I also provide experimental evidence

for the importance of these threatening processes, through laboratory trials of environmental tolerances.

2.2 Materials and methods

2.2.1 Study area

The South West Coast Drainage Division encompasses a land mass area of ~326000 km^2 and 19 river basins (Fig. 2.1). The south-west has a characteristic Mediterranean climate with hot dry summers and cool wet winters (Pen 1999). Most rainfall occurs in a narrow coastal zone and the temperate south-western tip (Fig. 2.2) where fertile forests, woodlands and heavily vegetated dunes dominate the landscape (Pen 1999; BOM 2011; Fig. 2.3). Further inland, the land is semi-arid to arid with less than 600 mm mean annual rainfall and dominated by dryland cropping (BOM 2011; Fig. 2.3).

2.2.2 Mussel distribution data

I compiled two databases of freshwater mussel occurrence in the South West Coast Drainage Division. First, an historical (pre-1992) database of 255 presence only records, obtained from museum specimen records and a few unpublished sources (See Appendix for details). Second, a current database containing 816 records of the presence or absence of *W. carteri* was compiled from museum data (19 records), site visits (see details below), between 2004 and 2011 (402 records), survey returns from the 'MusselWatchWA' web site (Klunzinger *et al*. 2009-2012 = 67 records) and 331 sites from unpublished data (Halse *et al*. 2002 = 27 records; Pinder *et al*. 2004 = 256 records; ARL 2006 = 1 record; Lymbery *et al*. 2008 = 31 records; Storer *et al*. 2011 = 7 records; WRM 2009 = 6 records: see Tables A2 – A4 for details). For 77 of the sites in the current database, I had records of mussel

presence from the historic database. For site visits (i.e. current presence or field surveys) within each locality (detailed in the appendix), I assessed whether freshwater mussels were present first by searching the banks for empty shells and visually from the water's surface when turbidity was low. This was followed by tactile searches of the sediments while wading or snorkelling in the river, lake or stream for up to one hour. If no live mussels could be found or if only empty shells existed in an advanced state of decay (worn thin and brittle), I assumed such sites as 'absence' sites. If the shells of closed mussels could not be pried apart, were observed with their siphons extended in the burrowed position, withdrew their foot when picked up or squirted water when disturbed, they were considered to be alive. Sites with living *W. carteri* were considered 'presence' sites.

2.2.3 Environmental predictor variables

I had 39 potential predictor variables available at the reach scale, derived from visual habitat assessments and chemical water parameters downloaded from the Western Australian Department of Water gauging station Water Resources Database (http://kumina.water.wa.gov.au/waterinformation/wrdata/wrdata.cfm). Given the importance of variable selection and the need to avoid over fitting the data during model construction (Burnham & Anderson 2002), I used a number of approaches to reduce the variable set. First, I divided the variables into categories of water habitat assessment, bank and substratum assessment and water chemistry. Within each category I then performed an exploratory principal components analysis and used factor loadings to identify correlated sets of variables. Rather than reduce the variable set to components, I preferred to choose a subset of predictor variables with potential functional relationships to physiological and behavioural attributes of the species (Leathwick *et al*. 2005; Elith & Leathwick 2009).

Where variables were highly correlated ($r > 0.6$), I first removed distal variables (e.g. rainfall) in favour of proximal variables (e.g. flow status) which are more likely to have a direct effect on species distribution (Wintle *et al*. 2005), and then removed single variables (e.g. ionic concentrations) in favour of composite variables (e.g. salinity). From this process, I was left with 11 environmental predictor variables (Table 2.1).

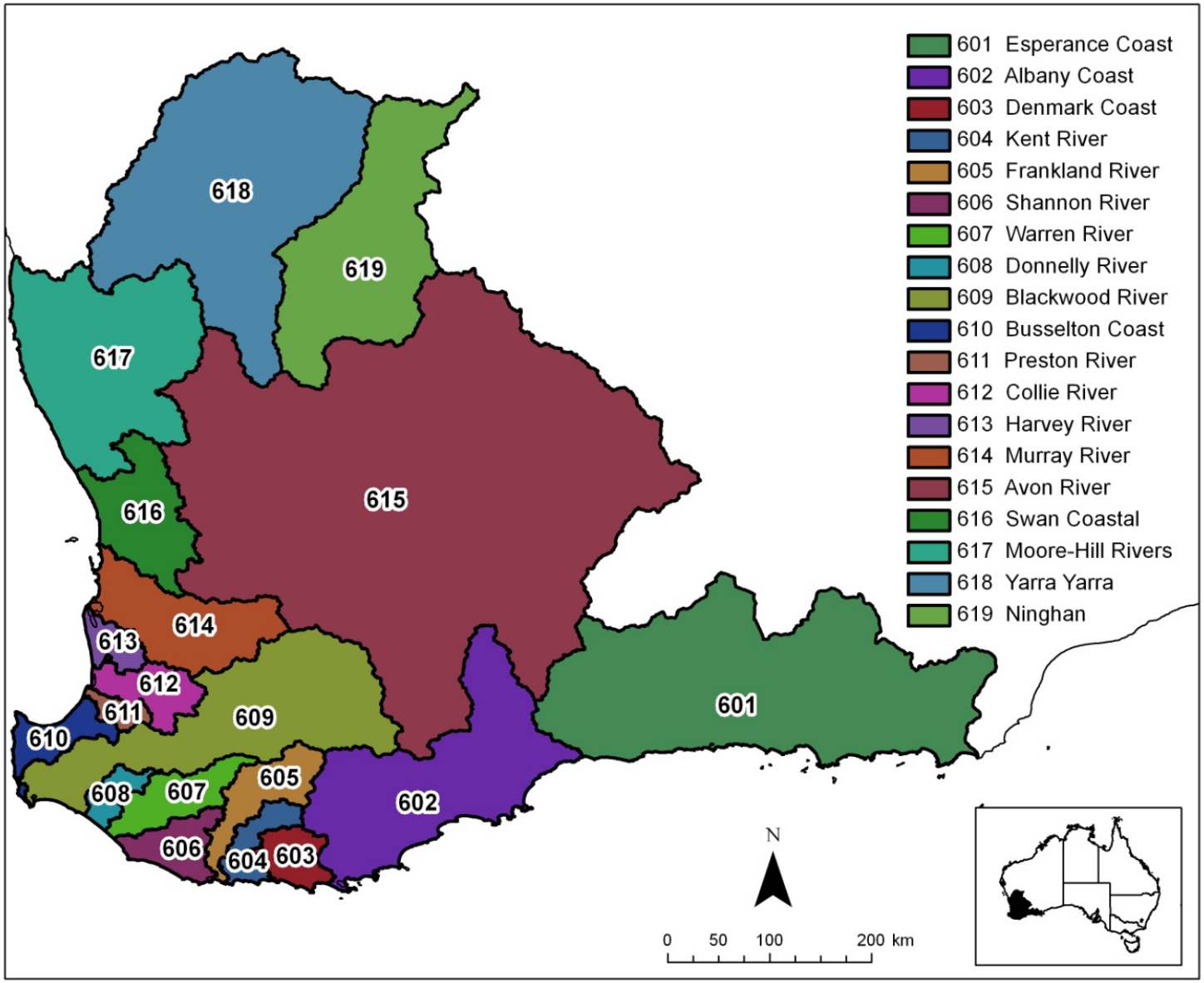

Fig. 2.1 River basins within the South West Coast Drainage Division of Australia. (Spatial data provided by Western Australian Department of Water, under license).

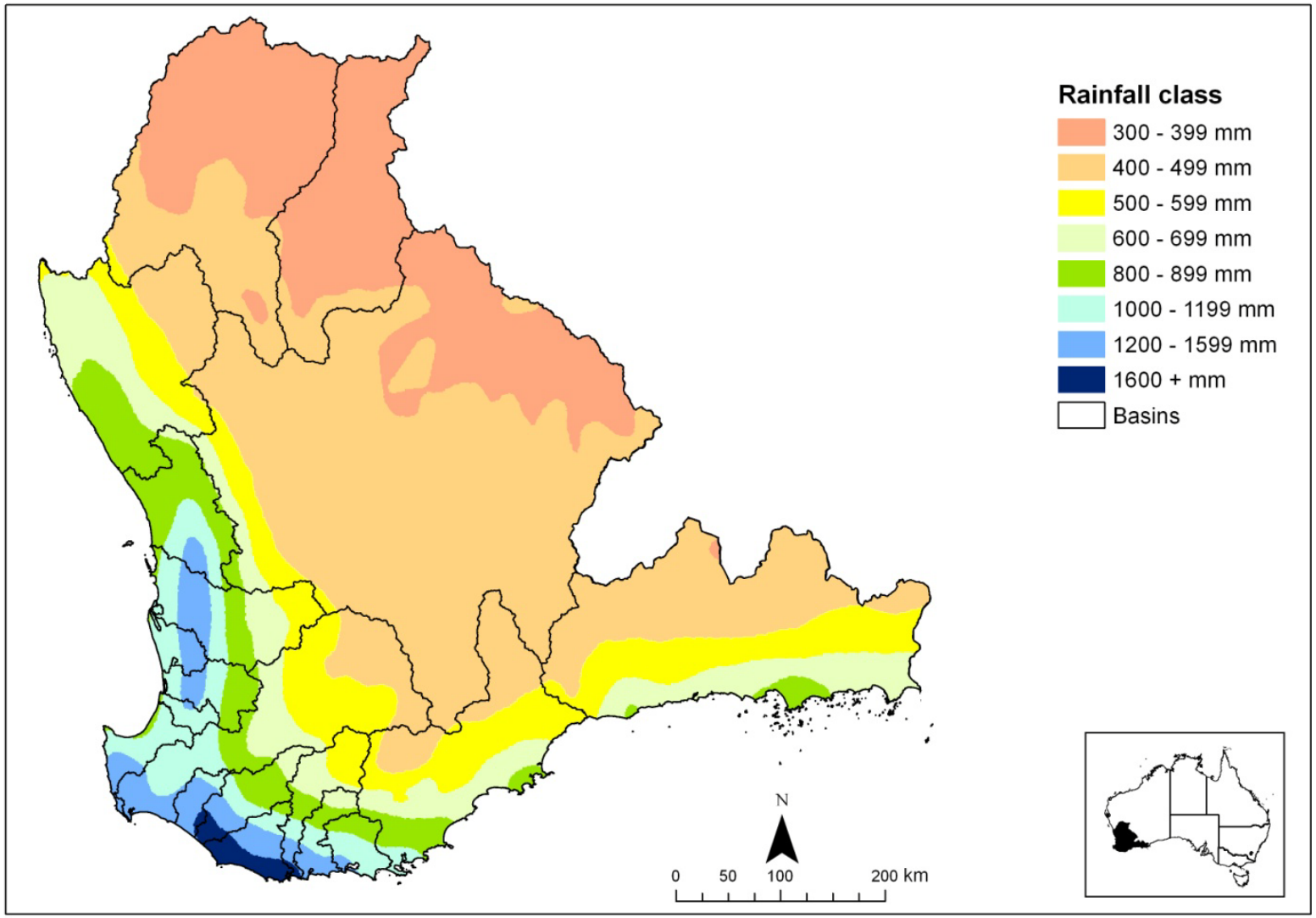

Fig. 2.2 Mean annual rainfall (1967- 2010) within the South West Coast Drainage Division of Australia. (Data provided by Western Australian Department of Water, under license).

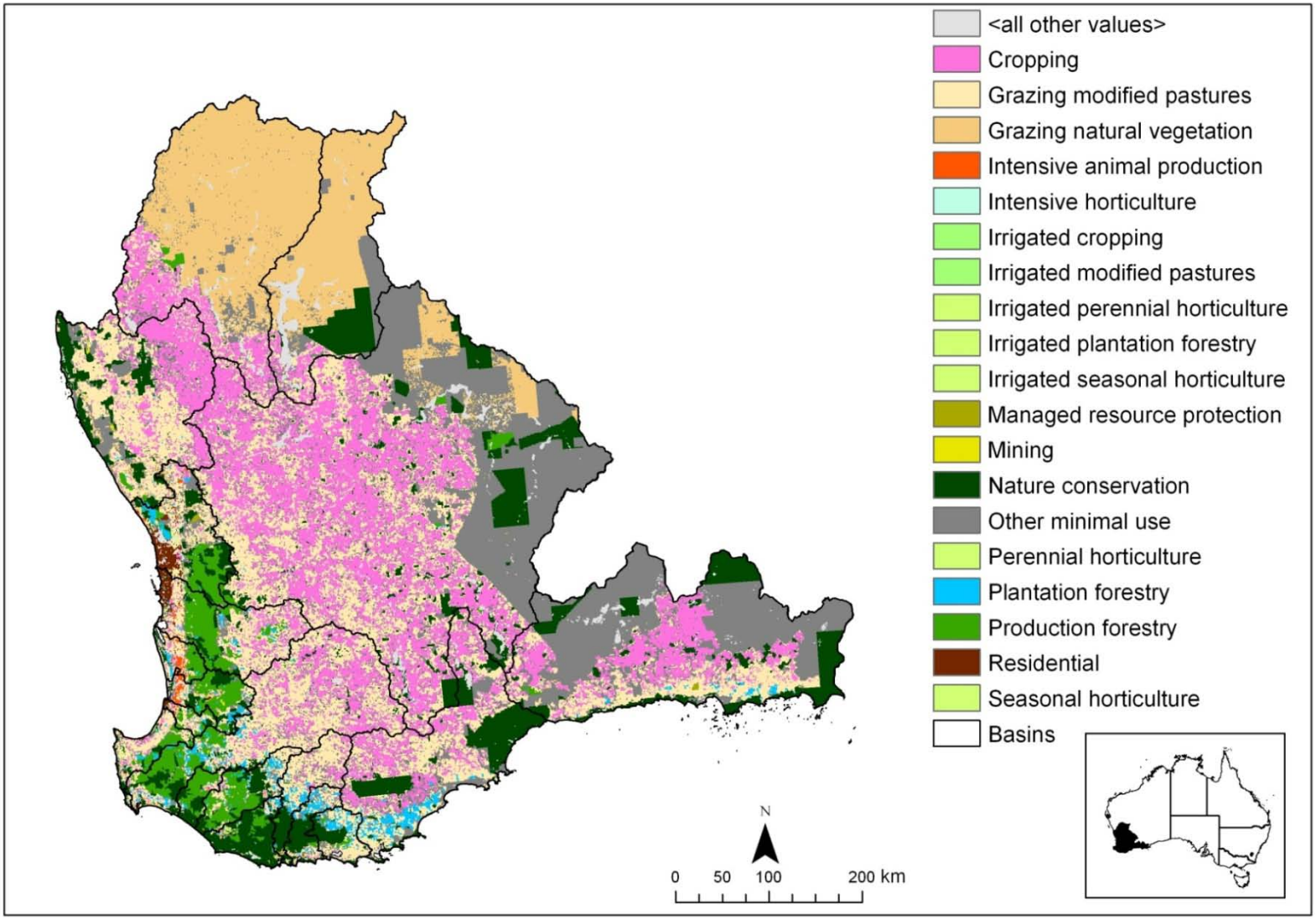

Fig. 2.3 Land use within the South West Coast Drainage Division of Australia. (Land use data from NLWRA 1997; spatial data provided by Western Australian Department of Water, under license).

Table 2.1 Environmental predictor variables used for analyses of factors controlling the distribution of *Westralunio carteri*

Variable name	Variable type	Description	Range	Mean (±s.e.)
Hydrology type	Nominal	1 = non-perennial 2 = perennial		
Water type	Nominal	1 = lotic; includes rivers, streams, creeks and springs 2 = lentic; includes swamps, lakes, pools, playas, lagoons, ponds and artificial and natural reservoirs		
Alkalinity (mg L^{-1})	Continuous	total carbonate alkalinity ($HCO_3^- + 2CO_3^{2-}$)	0 – 680	71.4 (3.8)
DO (%)	Continuous	dissolved oxygen (DO)	8.7 – 76.0	75.5 (1.1)
Hardness (g L^{-1})	Continuous	Total $CaCO_3$ {$Ca^+ K^+$)	0.0 – 47.0	2.5 (0.3)
pH	Continuous	pH $\log_{10} a_{H^+} = \log_{10}(\frac{1}{a_H})$, where a_H is hydrogen ion activity	2.1 – 10.5	7.3 (0.04)
Salinity (g L^{-1})	Continuous	total dissolved salts	0.0 – 13.9	0.05 (0.03)
TN (mg L^{-1})	Continuous	total nitrogen	0.04 – 9.4	1.1 (0.05)
TP (mg L^{-1})	Continuous	total phosphorus	0.01 – 3.1	0.2 (0.02)
Turbidity (NTU)	Continuous	Nephelometric Turbidity Units	0 – 520	14.2 (1.7)
Temperature (° C)	Continuous	degrees Celsius	10.5 – 35.2	16.9 (0.2)

2.2.4 Species distribution analysis

I mapped historic and current presence records as vector data in ArcGIS™ Desktop 10 using the GCS_GDA_1994 coordinate system on a 1:250000 scale Australian Geoscience Map Sheet Index (Geoscience Australia 2003). From the distribution point data, extent of occurrence (EOO) was determined by constructing minimum convex polygons (α-hulls) in ArcGIS™ Desktop 10, using IUCN guidelines (IUCN 2011). Two α-hulls were constructed from species distributions using the Triangulated Irregular Network (TIN) feature in ArcGIS™ Desktop 10; one was drawn for historic EOO and one was drawn for the current EOO of *W. carteri*. The areas of the resulting polygons were determined using the 'identify' feature and total area was summed for each dataset. The percentage change between the α-hulls thus allowed me to estimate the temporal change in EOO.

Current occurrence of *W. carteri* was related to environmental predictors using generalized linear model (GLM) and generalised additive model (GAM) approaches; very

similar results were obtained and only the GLM models are reported here. As my focus was to determine the relative importance of different environmental variables in explaining species distribution, rather than to obtain a predictive model, I employed the following stepwise approach. The full model was fitted and the statistical significance of each predictor variable tested by the difference in log-likelihoods of the full model and the model without the effect. Spatial autocorrelation of model residuals with straight-line distance between sites, was tested using Morans I (Dormann *et al*. 2007). Variables with $P < 0.10$ were then retained to produce a final set of models containing all combinations of the predictors, and these models were compared using an information-theoretic approach (Burnham & Anderson 2002). Akaike likelihood weights were calculated from Aikaike Information Criterion (AIC) scores for each model, models were ranked from best-fitting to worst fitting and summed likelihood weights were used to generate a 95% confidence set of best-fitting models (Whittingham *et al*. 2006). The relative importance of each environmental predictor variable was then determined by summing the Akaike likelihood weights across all models in the 95% confidence set which contained that variable; this gives the selection probability that a given variable will appear in the AIC-best model (Burnham & Anderson 2002).

To further investigate environmental predictors, I chose a subset of sites which all had perennial hydrology types (see Results) and for which complete datasets were available for the other variables (n = 280), and used Bray-Curtis ordination to relate mussel presence/absence to environmental variables using the relative Euclidean distance metric and default options in PC-ORD (v. 6: McCune & Mefford 2011). Finally, to explore the extent to which environmental predictors may be responsible for changes in mussel distribution, I compared current and historical mussel occurrence in those 77 sites for which

mussels occurred historically and for which I had current data. Changes in mussel occurrence were coded as 0 (historically present and currently absent) or 1 (historically and currently present) and related to environmental predictors using the GLM approach described above.

2.2.5 Salinity tolerance experiments

A total of 160 *W. carteri*, with shell lengths between 52 and 72 mm, were hand-collected from a mildly brackish site (salinities ranging from 0.39 to 3.16 g L^{-1} and a mean of 1.1 g L^{-1}) in the Collie River (33°18'1.36" S, 115°49'1.06" E). Additionally, 80 *W. carteri*, ranging in size from 68 to 87 mm long, were hand-collected from a site in Yalyal Brook (31°30'09" S, 115°59'47.39"E), which is a spring-fed creek with salinities less than 0.5 g L^{-1} (Beatty *et al*. 2010a).

Upon capture, *W. carteri* were placed in a 10 L plastic bucket containing river water and transported to the laboratory. Mussels were acclimated in freshwater (salinity < 0.5 g L^{-1}) aquaria with a recirculated filtration system for two days prior to commencing salinity tolerance experiments. Acute salinity tolerance experiments were conducted in 20 continuously circulated, aerated aquaria (54 L) with four replicates per salinity treatment in each experiment. In an initial experiment, I tested the survival of *W. carteri* from the Collie River when exposed to a broad range of salinity treatments which consisted of a control (~0.5 g L^{-1}), 5, 10, 15 and 20 g L^{-1}. This was followed by two experiments in which I quantified acute tolerance of both the Collie River and Yalyal Brook to a finer range of salinity concentrations. Treatments for both experiments consisted of a control (~0.5 g/L), 1, 2, 3 and 4 g L^{-1}, with *W. carteri* sourced from the Collie River in one experiment, followed by those sourced from Yalyal Brook.

Treatment solutions were prepared by dissolving synthetic sea salt (WA Salt Supply, Inc.) in de-chlorinated tap water and salinity concentrations determined using an Oakton™ PCD650 portable water testing meter. The ionic composition of salinised rivers in Western Australia varies geographically and seasonally, but is typically similar to seawater, except for deficient concentrations of potassium (Partridge *et al.* 2008). After the acclimation period, Hallprint™ flexible 8 x 4 mm yellow polyethylene numbered shellfish tags were super glued onto one valve of each individual mussel for identification and four mussels were allocated randomly to each experimental aquarium. By chemical analysis, the manufacturer provided a certificate of conformance, with composition of the product as containing < 0.5 mg L^{-1} Copper (Cu) and < 1 mg L^{-1} Iron (Fe) and did not contain any biocide. Treatment solutions, therefore, contained Cu and Fe concentrations below LC_{50} levels reported for other unionoids (Milam *et al.* 2005; USEPA 2007; Wang *et al.* 2007). Mussels were observed daily and removed when dead, which was indicated by gaping valves that did not close when prodded.

Logistic regression analysis was performed on the proportion of individuals that died in each salinity treatment for each experiment. This analysis determined the lethal concentration (LC), with LC_{50} and LC_{95} representing the salinity at which 50 or 95% mortality occurred upon termination of the experiment, respectively. The logistic regression curve was fitted by bootstrapping 1000 random samples, and the LC_{50} and LC_{95} values calculated according to the model:

$$P_S = 1/[1 + e^{-\ln 19(S - LC50)/(LC95-LC50)}], \quad (1)$$

where P_S is the proportion of mussels that died at each salinity concentration, and LC_{50} and LC_{95} are the salinity concentrations at which 50 and 95% of the total sample of mussels died, respectively. The bootstrapping of 1000 random samples also produced upper and

lower 95% confidence intervals (CI) of the parameters being tested. As experiments were conducted at different times for the different populations of mussels, we were not able to formally test differences among populations in LC_{50} or LC_{95} values. Instead, non-overlapping 95% CI's for LC_{50} and LC_{95} in different experiments were taken as evidence of significant differences in these parameters between populations of mussels (Kefford *et al.* 2004).

2.2.6 Drying tolerance experiment

Westralunio carteri (*n* = 350) of similar size were collected from the Collie River, placed in a 5 L plastic bucket containing river water and transported live to the laboratory. Mussels were maintained live in 45 L aquaria containing dechlorinated tap water with continuous aeration for 2 weeks to acclimatise to captivity. After acclimatisation, small (8 mm X 4 mm) individually numbered polyethylene shellfish tags were attached externally onto each mussel's shell using Superglue and the number recorded for individual identification.

Mussels were then randomly allocated into three treatments. For Treatment A (controls), mussels were maintained in aquaria as they had been during the acclimatisation period. Treatment B was set up to simulate a drying river and containers consisted of 250 L (0.5 m x 0.5 m x 1.0 m) plugged outdoor bathtubs mounted to 1.5 m star pickets, elevated 1 m off the ground, and filled with commercially available washed river sand to a depth of ca. 200 mm, filled with dechlorinated tap water to a depth of ca. 7 cm above the sand substrate. Treatment C was the same as Treatment B, but did not contain any water. Treatments were randomly allocated with five individual mussels for each of ten replicate bathtubs/aquaria per treatment. Temperature data loggers (HOBO Pendant® UA-002-64, Onset Computer Corporation, Bourne, MA 02532, USA) were deployed at the beginning of the experiment

and placed into Treatment A and within a large (90 mm long) empty shell of *W. carteri* in Treatment C. The data were downloaded when the experiment was terminated. Mussels were monitored for mortality by removing treatment replicates on days 5 and 10 and placing mussels into aquaria, which had been set up the same as Treatment A, but contained no other mussels; each group of mussels from Treatment B and Treatment C were placed into separate aquaria. Mussels were left overnight in the test aquaria and checked the following day for mortalities. Mortality was evidenced by floating and/or gaping shells. Survivors from Day 5 were placed back into their respective treatment replicate bathtubs and the procedure was repeated on Day 10.

2.3 Results

2.3.1 Mussel distribution

Westralunio carteri's current EOO is 16,011.9 km^2, a 63.3% reduction from its former (historic) extent of 43,579.8 km^2 (Fig. 2.4). From historic records, *W. carteri* once extended from the Moore River, inland to the Avon and Blackwood Rivers and bounded by the Bow River (Fig. 2.5).

The species has disappeared from 51% of 114 historic sites and the current distribution includes freshwater streams, rivers, reservoirs and lakes within 13 of the 19 river basins of the South West Coast Drainage Division. *Westralunio carteri* is bounded in the north by Gingin Brook southward to the Kent River, within 50-100 km of the coast, and two outlying populations found in the Goodga and Waychinicup Rivers (Fig. 2.6).

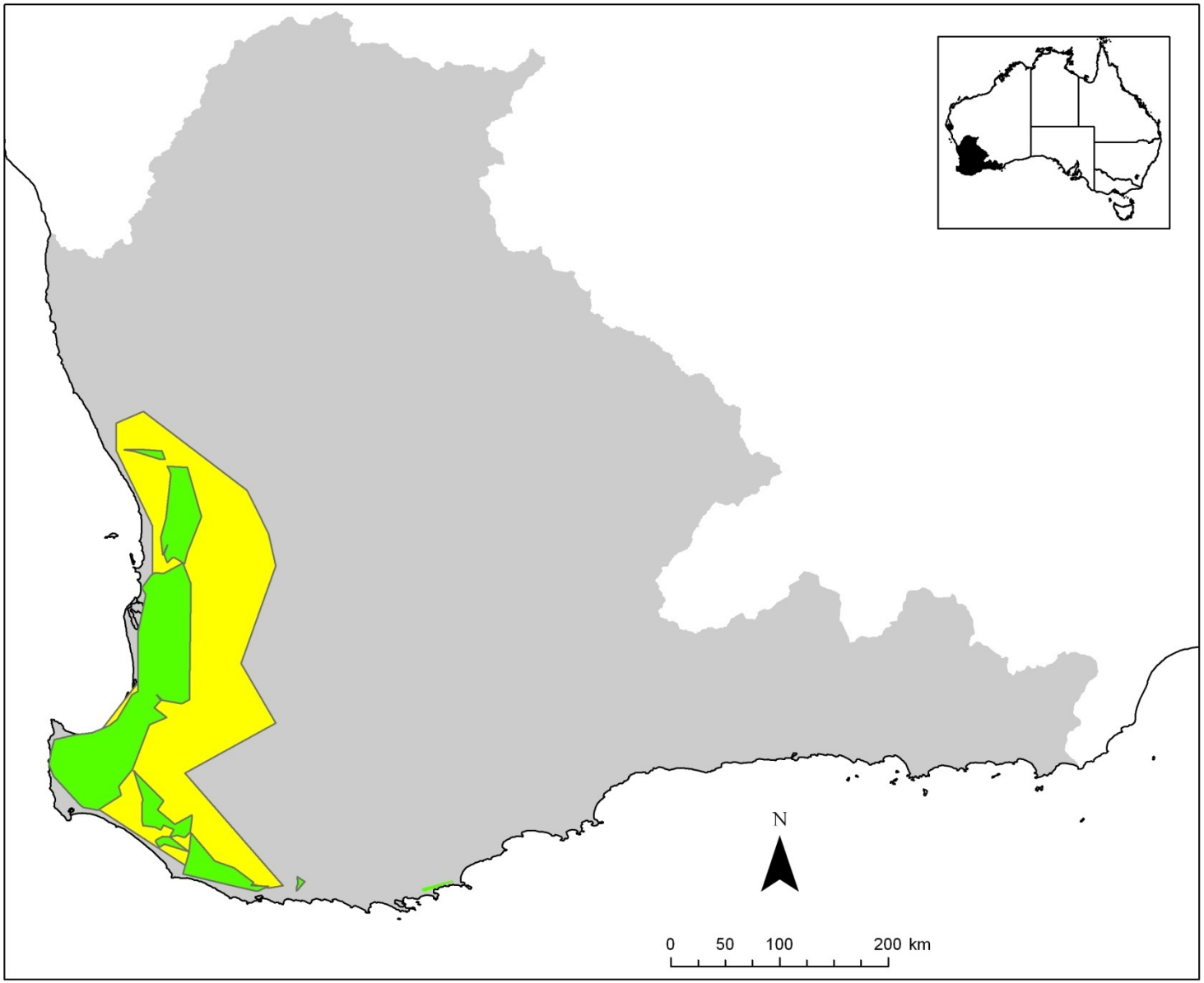

Fig. 2.4 Historic (yellow) and current (green) Extent of Occurrence (EOO) of *Westralunio carteri* within the South West Coast Drainage Division (shaded grey). (Spatial data provided by Western Australian Department of Water, under license).

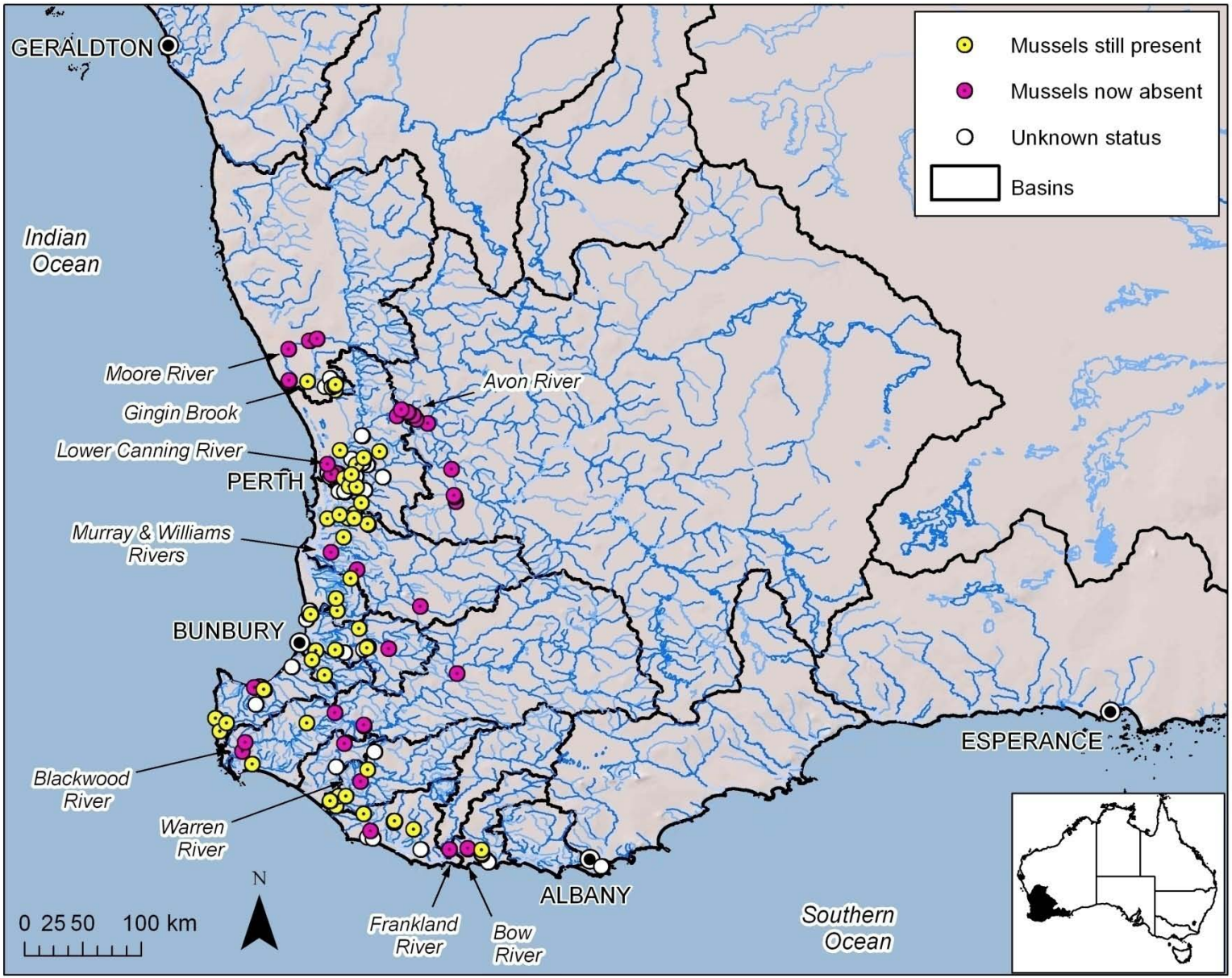

Fig. 2.5 Historic (pre-1992) distribution of *Westralunio carteri*. (Spatial data provided by Western Australian Department of Water, under license).

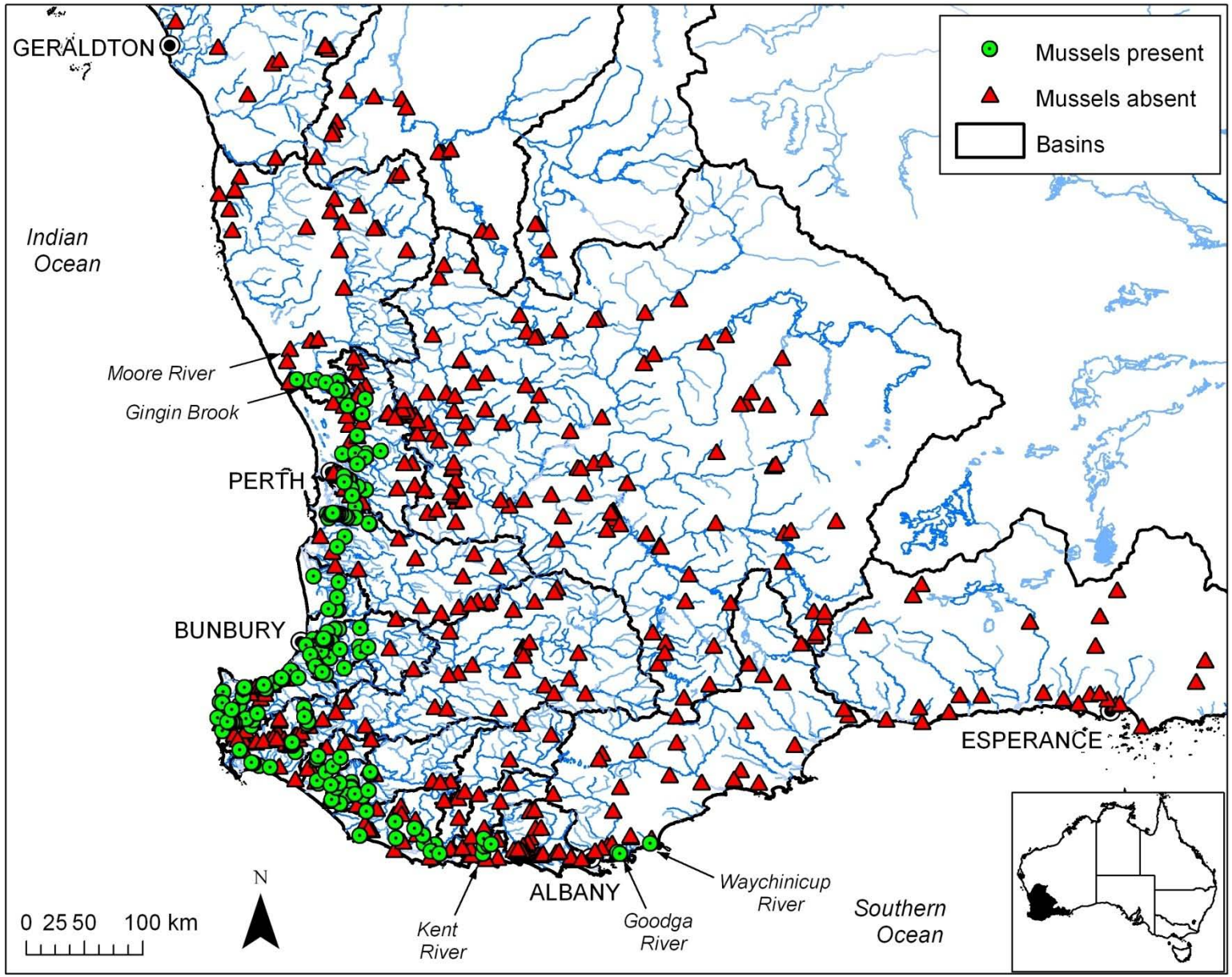

Fig. 2.6 Current distribution of *Westralunio carteri*. (Spatial data provided by Western Australian Department of Water, under license).

2.3.2 Environmental predictors of distribution

The full GLM model, containing all 11 environmental predictors, explained 30.5% of the variation in current mussel distribution. This suggests that other (non-measured) environmental factors may also be important, although there was no significant spatial autocorrelation of model residuals. Likelihood ratio tests indicated that only three variables had an effect on model fit with a probability < 0.10; hydrology type (P = 0.010), salinity (P = 0.062) and water type (P = 0.083). Of the six possible models containing all combinations of these three variables, only two were in the 95% confidence set, that containing hydrology and salinity (Akaike weight 0.73) and that containing all three variables (Akaike weight 0.27). The selection probabilities (i.e. the probabilities of being included in the best-fitting model) for hydrology type and salinity were therefore 1.0, compared to 0.27 for water type. With respect to hydrology type, mussels were never found in non-perennial water bodies, and were present in 53% of perennial water bodies that were surveyed. With respect to salinity, mussels were never found in water bodies with mean salinity greater than 1.5 g L^{-1} (Fig. 2.7).

In the subset of 280 sites from perennial systems for which there were no missing data, turbidity, salinity, TN and pH together explained ~90% (turbidity – 39.40%; salinity – 33.14%; TN – 9.63%; and pH – 3.73%) of the dissimilarity between distribution sites with and without mussels (Fig. 2.8).

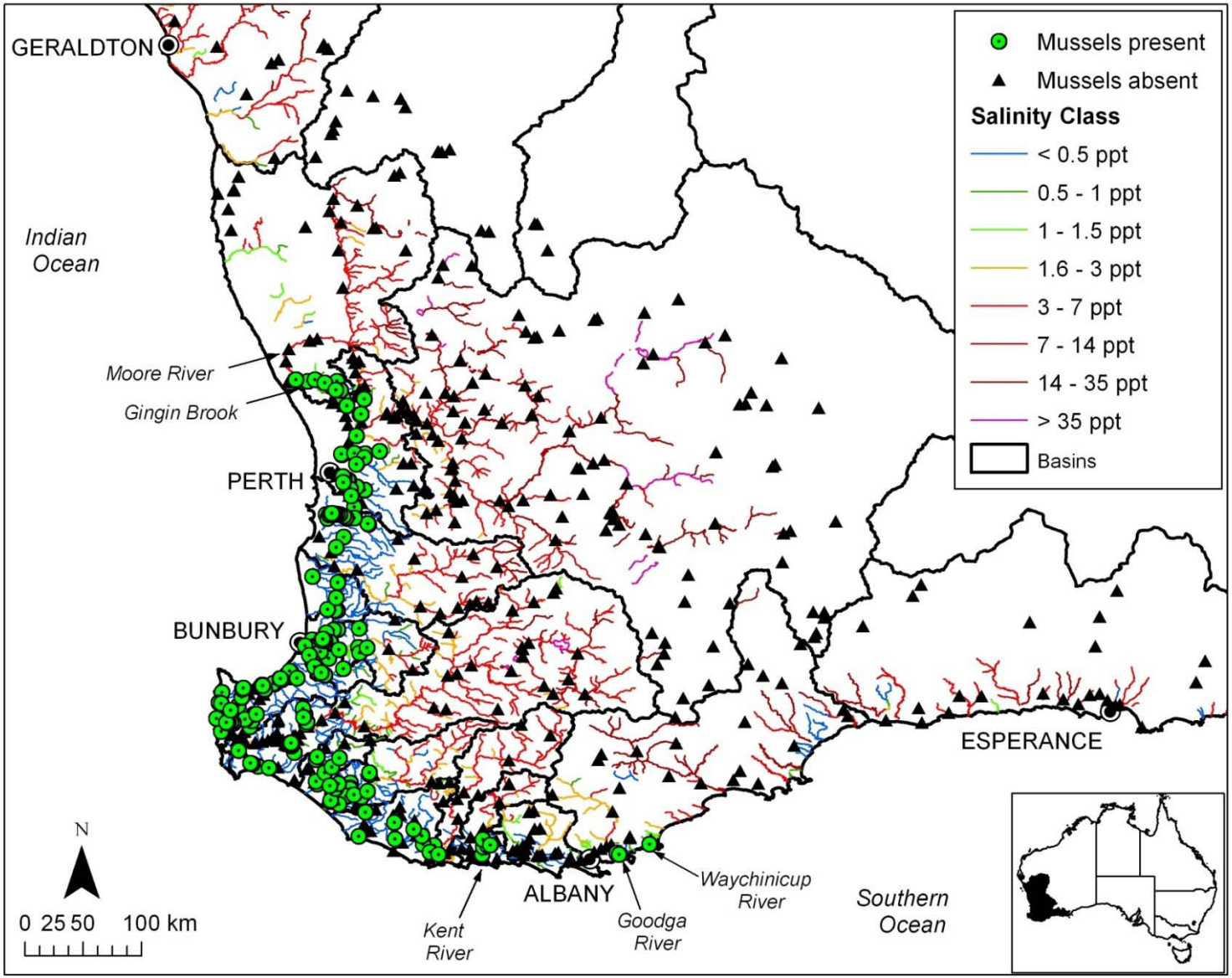

Fig. 2.7 Distribution of *Westralunio carteri* in relation to salinity (ppt = g L^{-1}) within south-western Australia. Salinity data (Mayer *et al*. 2005) and spatial data were provided by the Western Australian Department of Water under license.

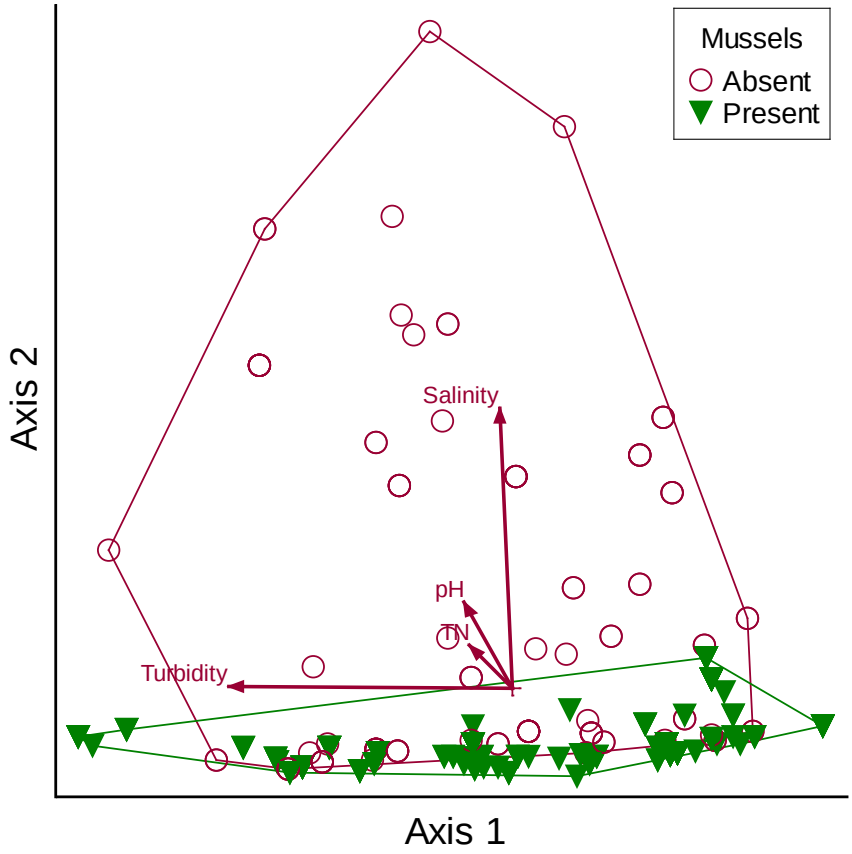

Fig. 2.8 Two-dimensional Bray-Curtis ordination (Relative Euclidean distance) of environmental prediction data for the distribution of *Westralunio carteri* within perennial sites with full datasets. Stress = 0.20. Vectors radiating from the data centroid indicate the relative strength of the coefficient of determination (r^2).

For the 77 historic sites in which I could examine changes in mussel distribution, a number of environmental predictors were either invariant or missing, leaving only temperature, salinity, turbidity, pH, TN and TP. The full model with these six predictors explained 92.6% of the variation in changed distribution, but the only variable with a significant effect on model fit was salinity ($P < 0.0001$); for all other variables $P > 0.99$. The mean salinity of sites at which mussels were historically present but are now absent was 5.65 g L^{-1} (SE 0.48 g L^{-1}), compared to a mean salinity of 0.37 g L^{-1} (SE 0.30 g L^{-1}) for sites where mussels were historically present and are still found (Fig. 2.9).

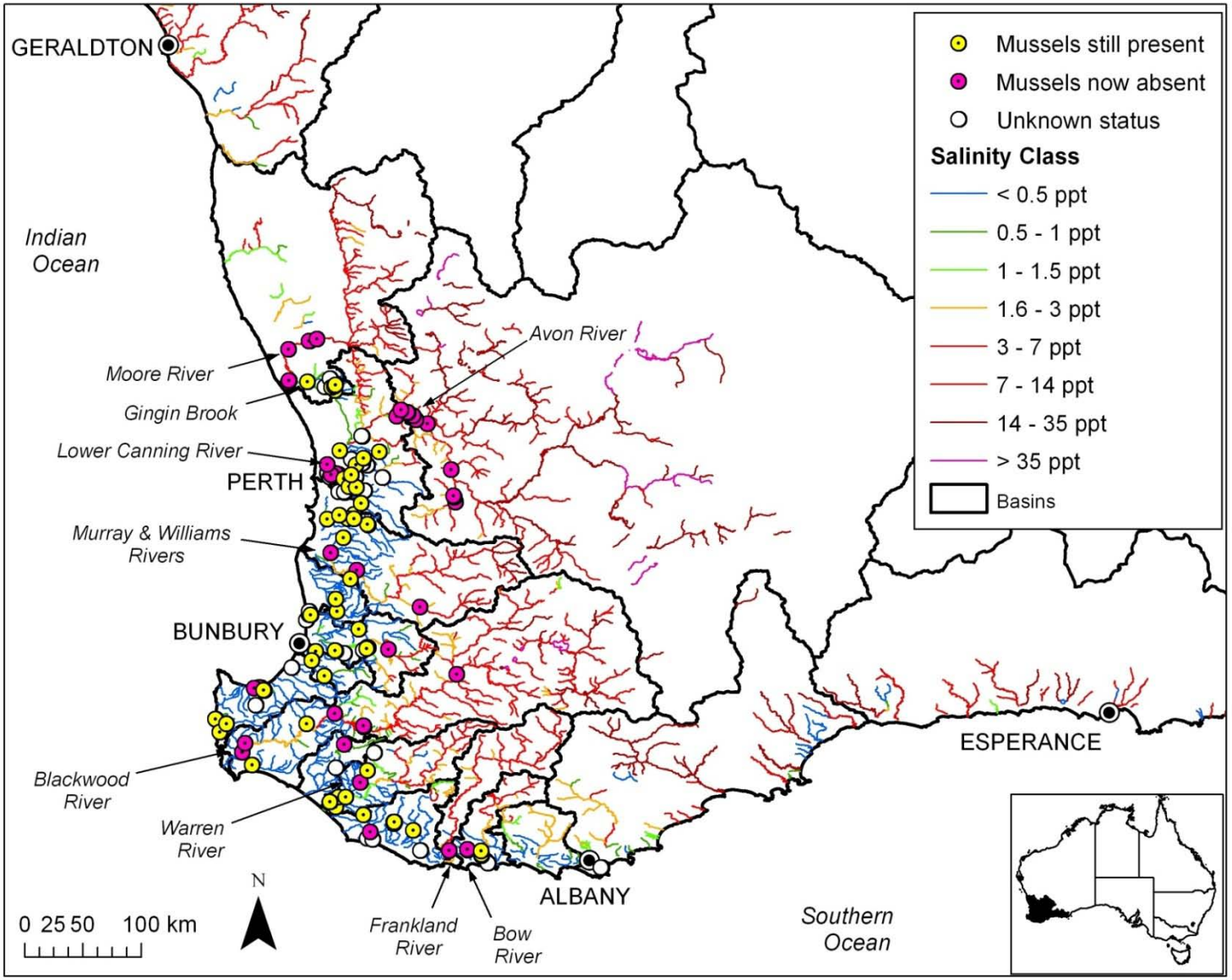

Fig. 2.9 Historic (pre-1992) distribution of *Westralunio carteri* in relation to salinity (g L^{-1} = ppt). Salinity data (Mayer *et al.* 2005) and spatial data were provided by the Western Australian Department of Water under license.

2.3.3 Salinity tolerance treatments

Survivability of mussels (from the Collie River) exposed to salinity concentrations of 0, 5, 10, 15 and 20 g L^{-1} over a 30 day period is given in Fig. 2.10a. No mortality was observed in control groups. In the 5 and 10 g L^{-1} treatments, the first incidence of mortality was observed on Day 6 and all mussels had died within eight days. In the other two treatments (15 and 20 g L^{-1}), actual mortality was not observed until Day 7 when valves began to gape. Valves in these mussels remained closed for the first seven days of the experiment, but upon examination on Day 7, mussels were foul-smelling, indicating autolysis and they had been dead for some time. Because salinities of 5-20 g L^{-1} were lethal to 100% of mussels, two more experiments were conducted on different populations of *W. carteri* to determine the effects of salinity in the 1-4 g L^{-1} range.

In the second experiment, no mortality was observed in mussels from the Collie River exposed to salinities of 1 and 2 g L^{-1} treatments, and only one mussel died at 0 g L^{-1}. Mortality was first observed in the 3 g L^{-1} treatment after 12 days and last observed on Day 23, with a cumulative mortality of 38%. Mortality was first observed after 10 days in the 4 g L^{-1} treatment, with 100% mortality by Day 28 (Fig. 2.10b).

For *W. carteri* sourced from Yalyal Brook, although a few mussels were lost in the 0 and 1 g L^{-1} treatments within the first few days of the experiment, no further mortalities were observed for the duration of the experiment. Mortality was first observed in the 2 g L^{-1} treatment after 2 days and last observed on Day 19, with a cumulative mortality of 43.7%. Mortality was first observed after 2 days in the 3 g L^{-1} treatment and after 3 days in the 4 g L^{-1} treatment, with 100% mortality by Day 11 (Fig. 2.10c).

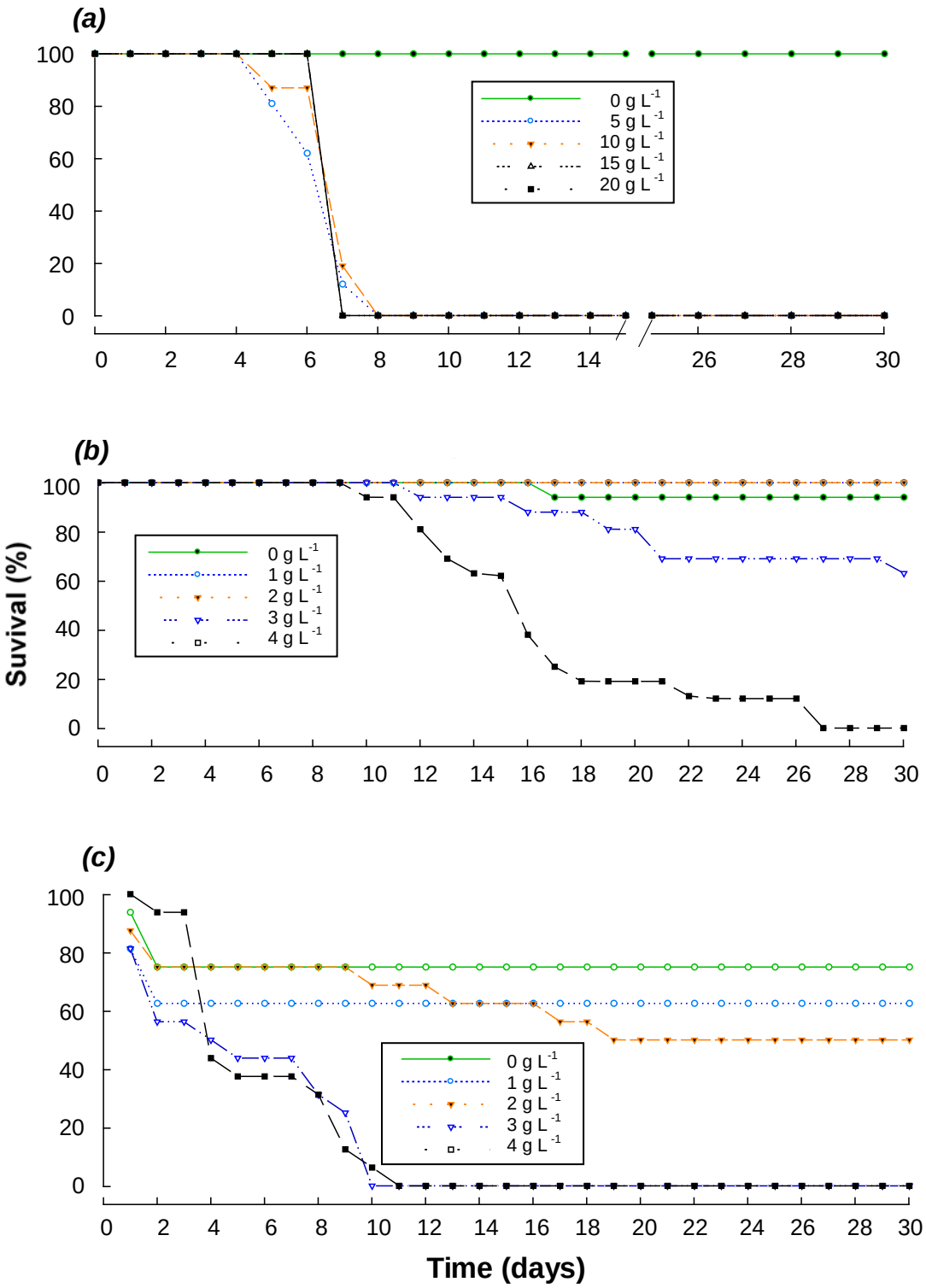

Fig. 2.10 Survival rates of *Westralunio carteri* exposed to varying concentrations of salinity (0, 1, 2, 3, 4, 5, 10, 15 and 20 g L^{-1}). *Westralunio carteri* sourced from *(a)*, *(b)* the Collie River and *(c)* Yalyal Brook.

2.3.4 LC_{50} and LC_{95}

The logistic regression curves with percentage mortalities over the fine scale salinity range (0 – 4 g L^{-1}) in the two populations of *W. carteri* are presented in Fig. 2.11. From the logistic regression curves, the LC_{50} value for *W. carteri* from the Collie River is 3.04 g L^{-1} (95% CI 2.80 – 3.30 g L^{-1}) and the LC_{95} value was 4.25 g L^{-1} (95% CI 3.13 – 5.47 g L^{-1}). The LC_{50} and LC_{95} values for *W. carteri* sourced from Yalyal Brook were 1.29 (95% CI 0.74 – 1.80 g L^{-1}) and 3.57 g L^{-1} (95% CI 2.87 – 4.38 g L^{-1}), respectively. Non-overlapping confidence intervals suggest that LC_{50}, although not LC_{95}, was greater for the Collie River population than for the Yalyal Brook population of *W. carteri*.

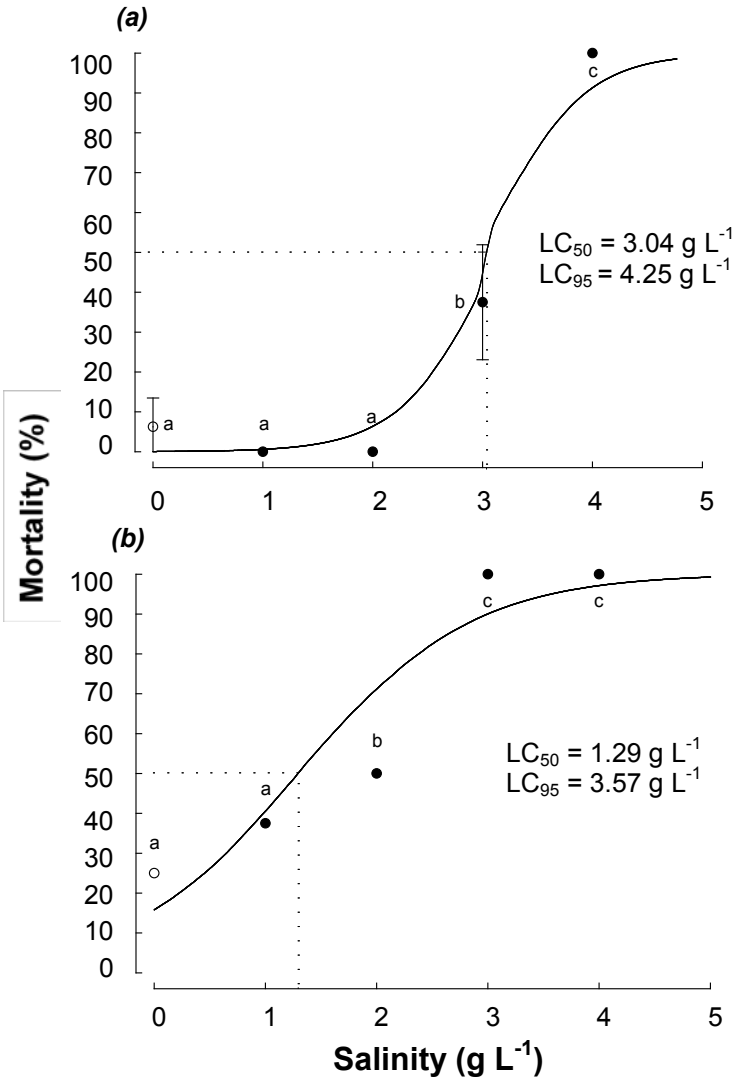

Fig. 2.11 Percentage mortalities over salinity treatments (0, 1, 2, 3, and 4 g L^{-1}) of *Westralunio carteri* from *(a)* Collie River and *(b)* Yalyal Brook. Logistic curves (including 95% confidence limits from the logistic regression analysis are provided. Different letters indicate significant difference between treatments ($P < 0.05$).

2.3.5 Drying tolerance experiment

No mussels in the control groups (Treatment A) died at any time during the duration of the experiment. By Day 3 of the experiment, no standing water remained in Treatment B; however, the sand in this treatment was still moist when mussels were observed on Day 5. There were significant differences among treatments in five day mortalities (F = 896.19; df = 2, 21; P <0.001) as well as 10 day mortalities (F = 752.74; df = 2, 21; P < 0.001). Pair wise comparison of treatment groups in both the five day and ten day sampling periods showed that mean mortalities between all treatments were significantly different (Tukey's tests, P < 0.03). On Day 5 of the experiment, 76% of the 100 mussels in Treatment B had died and 99% of the 100 mussels in Treatment C were dead. After ten days, 94% of 100 mussels were dead in Treatment B and there were no survivors in Treatment C (Fig. 2.12).

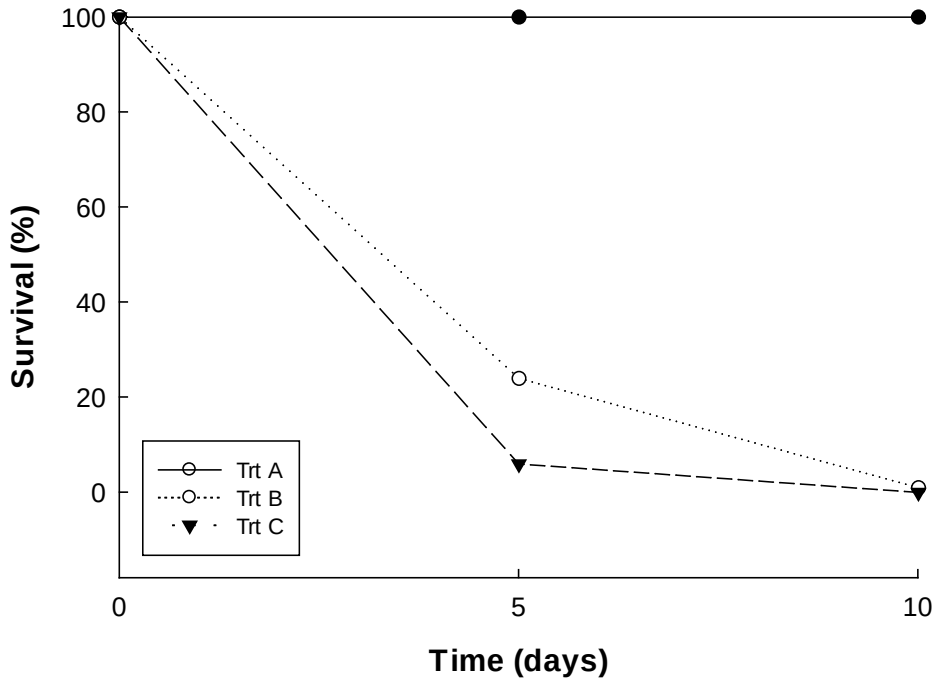

Figure 2.12 Survival of *Westralunio carteri* on Days 5 and 10 of a controlled dehydration exposure experiment. Treatments: A = controls, mussels maintained in aerated aquaria; B = mussels placed in 250 L outdoor bathtubs containing river sand (depth ca. 200 mm) with dechlorinated tap water (depth of ca. 70 mm above the sand); C = mussels placed in 250 L outdoor bathtubs containing dry river sand, without water.

Temperature within the empty mussel shell placed in Treatment C ranged from a daily maximum of 65° C to an overnight daily low of 8.2° C and a mean temperature of 29.4° C. Within Treatment A, maximum daily temperature reached 24.8° C with a minimum recorded overnight low of 20.3° C and a mean temperature of 22.6° C.

2.4 Discussion

2.4.1 Conservation status

In this study, I have provided the first comprehensive assessment of the distribution of *W. carteri*, the only freshwater mussel found in the South West Coast Drainage Division of Australia. Because Area of Occupancy (AOO), generation length and population size cannot presently be determined due to a lack of data, these criteria should not be considered in the conservation assessment of *W. carteri*.

However, EOO was effectively determined from a wealth of current and historic presence and absence data. Using IUCN Red List guidelines (Guideline 4.9, pp. 31-34: IUCN 2011), the difference between the historic and current EOO indicates that *W. carteri* has undergone a 63.3% range reduction from its former extent within less than 50 years. In addition, the species has disappeared from 51% of sites where it was formerly known to exist. This suggests that *W. carteri* should be listed as 'Endangered' under criteria A1 + 2(c): "Population reduction observed, estimated, inferred, or suspected in the past where the causes of reduction may not have ceased OR may not be understood OR may not be reversible, based on (a) to (e) under A1"; "(c) a decline in area of occupancy (AOO), extent of occurrence (EOO) and/or habitat quality)" and "≥50%" decline in EOO from the IUCN Red List Guidelines (IUCN 2011). Furthermore, *W. carteri* could qualify as 'Vulnerable' under criteria B1 (a) + (b)(i) and (iii) where B1: "Extent of Occurrence (EOO) <20,000

km^2"; (a): "Severely fragmented OR Number of locations ≤10" (where 'location' is "a geographically and ecologically distinct area in which a single threatening event can rapidly affect all individuals of the taxon"; here we define river basin as location and the threat as salinity) and (b): "Continuing decline in..." (i) "extent of occurrence" and (iii) "area, extent and/or quality of habitat".

The results of the current study clearly place into question previous listings of the conservation status of *W. carteri*, initially as Vulnerable (IUCN 1996), and more recently re-assessed as Least Concern (Köhler 2011). The original listing as Vulnerable was based on the species' disappearance from the Avon (Kendrick 1976) and Blackwood Rivers (Williams *et al.* 1991) as a result of salinisation, but was not based on a comprehensive assessment of the range of *W. carteri* and did not consider the full extent of the threat posed by salinisation or other environmental factors. The recent change in conservation status of *W. carteri* from Vulnerable to Least Concern was based on the assessment that the species is 'widespread' and 'abundant' elsewhere other than the Avon River in Western Australia from Bennet-Chambers *et al.* (1999) and Sommer *et al.* (2008), yet these authors only mention the species from the Canning River and the Gnangara Mound, near Perth, WA and do not give a full account of the species distribution. The assessment also states that the species is tolerant of human disturbance and organic pesticides, but this is a subjective interpretation of Storey & Edward (1989) and Bennet-Chambers *et al*. (1999) given that these studies were not toxicological in nature and no sub-lethal effects of the bio-accumulated chemicals were tested. The lack of published information on the species in terms of quantified distribution, abundance, tolerance to various environmental factors and salinity as well as the recent *ad hoc* assessment, and given that original nomination as 'Vulnerable' (A1c, B1 + 2bc), despite the absence of rigorous supporting information,

places the species current conservation status in question and is a good example of why IUCN criteria should be reviewed carefully during the assessment process. Given that there was clearly not enough information to adhere to the guidelines in both the original (IUCN 1996) and current (Köhler 2011) listings, a change to 'Data Deficient' may have been more appropriate in both cases.

2.4.2 Threats

An important component of the assessment of conservation status for a species should be the identification of threats which will guide future management actions (IUCN 2011). The distribution modelling suggests that the current EOO of *W. carteri* is determined, to a large degree, by perennial water availability and salinity concentration. Mussels were more likely to be present in perennial water bodies, particularly in rivers, and at low salinity. Other environmental factors such as pH and TN may have some effect on distribution but appear to be of lesser importance.

Turbidity

Turbidity appeared to be an important predictor of *W. carteri* distribution within perennial systems, slightly more so than salinity when only perennial sites were considered. Aldridge *et al*. (1987) showed that exposure to high concentrations of suspended solids and turbulence negatively impacted filtration ability in three European freshwater mussel species. Increased turbidity has been reported as being the strongest descriptor of reduced invertebrate density and biomass in North America (Wagener & LaPerriere 1985; Henley *et al*. 2000). Turbidity is often used as a surrogate measure for sediment and particulate matter overloading in aquatic environments, which can arise from inorganic and organic

sources, which can include dissolved salts (Newcombe 1994; Newcombe & Jansen 1996). Jones & Byrne (2010) reported sedimentation (and thus turbidity) as a major threatening process for freshwater mussels in south-eastern Australia.

Salinisation

A unique aspect of the current study was the ability to infer environmental factors responsible for past range reductions of *W. carteri*, by re-sampling sites at which mussels had previously been present from museum records. Salinity, as well as being an important predictor of current distribution, was by far the most important variable explaining the reduction in EOO from historic levels. The importance of salinity is therefore a major threatening process for the species and was further confirmed by salinity tolerance experiments, which showed that *W. carteri* was sensitive to acute changes in salinity, with salinities of approximately 4.1 g L^{-1} lethal to 95% of mussels and salinities of 3.1 g L^{-1} lethal to 50% of mussels tested from the Collie River. Those tested from Yalyal Brook, which is a much fresher system, were less tolerant, with salinities of approximately 3.6 g L^{-1} lethal to 95% of mussels and salinities of 1.3 g L^{-1} lethal to 50% of mussels tested. This suggests there may be some level of adaptive salinity tolerance in the Collie River, which has gradually become more salinised in recent history (Mayer *et al*. 2005), although further studies are needed to confirm this.

The salinity tolerance levels I found for *W. carteri* are similar to other reports which suggest that Australian hyriids are generally restricted to an upper salinity tolerance of 3.5 g L^{-1} (Walker 1981; Walker *et al.* 2001). My results suggest that salinity levels in excess of 3 g L^{-1} will seriously affect the abundance of *W. carteri* populations, and levels greater than 4 g L^{-1} may lead to local extirpation. In the field, *W. carteri* was never found in rivers with

salinity greater than 1.5 g L^{-1} and my laboratory experiments may have overestimated the salinity tolerance of the species. I tested only adult mussels and it is possible that larval and juvenile stages are more sensitive to increasing salinity, as is found in other species of invertebrates and vertebrates (James *et al.* 2003). In Walker (1981), for example, survival of the larval stages of *Alathyria jacksoni* IREDALE, 1934 and *Velesunio ambiguus* (PHILIPPI, 1847 decreased from 13 days at 0.4 g L^{-1} salinity to about half a day at 6 g L^{-1} salinity. Future research is necessary to determine the salinity tolerance of different life cycle stages and different populations of *W. carteri* so that we can more accurately predict the impact of secondary salinisation on the distribution and abundance of the species.

Secondary salinisation of streams and rivers is a major environmental problem in Australia. It has been estimated that 11,300 km (roughly 5%) of Australia's rivers are currently salinised (i.e. have a mean annual salinity of > 5 g L^{-1}) (ANZECC 2000; Lake & Bond 2007). It is expected that the salinity of freshwater ecosystems in many parts of Australia will continue to increase, mainly as a result of past land-use practices, with salinisation of up to 41,300 km of rivers and the mean salinity in Australian freshwater ecosystems increasing from the current level of less than 0.5 g L^{-1} to as much as 10 g L^{-1} by 2050 (NLRWA 2001; James *et al.* 2003; Lake & Bond 2007). The south-west corner of Western Australia is a hot spot in terms of secondary salinisation. More than 70% of Australia's salinisation occurs in this region (NLWRA 2001; Halse *et al*. 2003). As a result, most of the freshwater ecosystems in the south-west are already affected by increasing salinity, with 56% of the flows in the 30 largest rivers being brackish or saline and the trend expected to continue (Mayer *et al.* 2005).

In conjunction with the decline of *W. carteri* from systems affected by salinisation, Kendrick (1976) reported the colonisation of formerly freshwater habitats in the Avon

River by the estuarine bivalve *Fluviolanatus subtorta* (DUNKER, 1857) (Bivalvia: Trapeziidae), which was followed by similar references to this trapezid colonising salinised areas of the Blackwood River (Pen 1999). During my field surveys, I would occasionally find this species in salinised sites where *W. carteri* was absent including the Moore, Lower Canning, Blackwood and Frankland Rivers. I have also observed this species and *Mytilus* (*Mytilus*) *planatus* LAMARCK, 1819, a marine/estuarine mytilid, inhabiting the salinised Kalgan River nearly 40 km inland from the estuary where *W. carteri* had once been found.

What became evident during field surveys was the importance of freshwater refuges in salinised catchments, which harboured populations of *W. carteri*. Several of these included tributaries such as Yalyal Brook, Breera Brook, Lennard Brook, Wooroloo Brook, Jane Brook and Bennett Brook in the Swan Coast Basin and Milyeannup Brook and St. John Brook in the Blackwood River catchment. Their small size in terms of discharge, their heavy dependence on groundwater discharge to maintain habitats during summer, stream width, depth and length along with their isolation from the main rivers suggested to me that they might be more vulnerable to other threats that may not have been as common in the wider, deeper and longer main channels, as has been suggested for *Nannatherina balstoni* REGAN, 1906, a vulnerable salt-sensitive freshwater fish restricted to freshwater tributaries of the salinised Blackwood River, which depend heavily on fresh groundwater discharge during base flow periods (Beatty *et al.* 2010b; Beatty *et al*. 2011; Morrongiello *et al*. 2011).

It is important to have knowledge of the seasonal variations that can occur in salinity as well. Rivers connected to the highly salinised Avon Wheatbelt bioregion (see McKenzie & May 2002 for bioregions) receive large pulses of salt during winter flow periods, which may not be evident if sites are freshened by sporadic rainfall events during

baseflow periods, which is known to occur in the Avon, Swan, Blackwood, Murray, Warren and Kent Rivers, for example (Mayer *et al*. 2005; CSIRO 2009a,b). In higher rainfall areas of the south coast, lower reaches of the Warren, Kent, and to some extent the Donnelly River are maintained as fresh to marginal ($\leq$ 1 g L^{-1}) by significant freshwater flows entering from tributaries, whereas upper reaches can be highly brackish or saline (1.5 to > 3 g L^{-1}). In the Warren River, I found live *W. carteri* restricted to within 1 m of the bank, but all mussels in the middle channel were dead, suggesting that localised rainfall and groundwater intrusion may have stratified the salinity gradient and maintained localised colonies of mussels but not in salinised flows of the mid-channel during winter high flow periods when salt washes down from upstream. Populations within these rivers could potentially be at risk if salinity continues to rise and rainfalls decrease. During base flow periods, the Warren River is maintained with low salinities from fresh groundwater discharge, as is the case in the Blackwood River. Thus, ensuring groundwater extraction does not result in the loss of these freshwater refuges and access to freshwater habitats in otherwise salinised catchments will be crucial to the survival of freshwater fauna of the region (Beatty *et al*. 2010b, 2011) and particularly for *W. carteri*, which is up to five times more salt-sensitive than the freshwater fishes which have been tested (Beatty *et al*. 2011).

Salinity problems have also resulted from increased tides from sea level rise coupled with reduced flows of freshwater which have caused 'salt wedges' to move upstream from the Swan-Canning Estuary and caused 100% mortality in February 2011, resulting in a localised extirpation of *W. carteri* along a 8.5 km section of the Lower Canning River and one of its tributaries (Yule Brook) (see Klunzinger & Lymbery 2012; Klunzinger *et al.* 2011b). Similarly, Hastie *et al.* (2003) suggested that populations of

Scottish freshwater mussels are at risk of being immersed in seawater in the lower reaches of rivers within the next 40 years due to predicted sea level rise.

Drying

The other major threat to the conservation of *W. carteri*, as identified from species distribution modelling, is seasonal drying of habitat. While some rivers and streams in south-western Australia (e.g. Gingin Brook, Milyeannup Brook, Yalyal Brook) are maintained by springs and groundwater discharge in late summer-autumn, others are subject to loss of flow and extreme drying. This has a major influence on the ecology of freshwater organisms, which must either aestivate or migrate to permanent pools if they are to survive the dry season (Pen 1999). *Westralunio carteri* was only found in perennial systems, suggesting the species does not aestivate as previously thought (Storey & Edward 1989), unlike other aquatic fauna of the region which have the ability to do so (e.g. Burbidge 1981; Allen & Berra 1989; Withers 1998; Galeotti *et al*. 2010). Although I occasionally found *W. carteri* inhabiting mud burrows where water had receded, habitats were generally well shaded and sediments remained moist. Rarely did I find the species in headwaters of streams which dried in summer. Wherever I found *W. carteri* on exposed mud, baked hard in the sunshine, either the shells were empty or they had recently died.

The laboratory experiment demonstrated that *W. carteri* that were exposed to direct sunlight and heat with no shade and little to no available moisture cannot survive. There have only been a few documented studies on freshwater mussels' response to drought. In North America, for example, where freshwater mussels were exposed to record drought conditions, mortality rates ranged from 14-90%, depending on species and habitats available. The presence of woody debris, shade, cooler groundwater inputs and the ability

to burrow into moist sediments assisted in freshwater mussel survival (Miller & Payne 1998; Golladay *et al*. 2004; Gagnon *et al*. 2004; Haag & Warren 2008), and has also been observed for *V. angasi* in northern Australia (Humphrey 1984). From the little amount of information available, some Australian Hyriidae, such as *Velesunio ambiguus* (PHILIPPI, 1847) and *Velesunio angasi* (SOWERBY, 1867) may be well-adapted to aestivation for extended periods, particularly those occurring in temporary floodplain billabongs and ephemeral streams in remote inland areas (Walker 1981; Humphrey 1984; Sheldon & Walker 1989). The main consideration in survival is the mussel's capability to utilize anaerobic respiration. Ch'ng-Tan (1968) found that *V. ambiguus* survived for one year out of water. Walker *et al*. (2001), however, notes that Australian hyriids are not widespread in ephemeral or salinised water.

Rainfall in south-western Australia has fallen by 15% since the 1970s; mean annual stream flow into Perth reservoirs was 338 GL from 1911 to 1974, reduced to 177 GL yr^{-1} between 1975 and 2000 and to 75 GL from 2001 to 2010 (DCCEE 2010). Rainfall is expected to reduce by 7% and surface water runoff by 14% from 2021 to 2050 and if current trends continue, drought months will increase by 80% by 2070. Coupled with a rising demand for freshwater resources (including groundwater extraction) to feed a growing Perth population this trend is expected to severely impact endemic species (DCCEE 2010), including freshwater fishes (Beatty *et al*. 2010b, 2011; Morrongiello *et al*. 2011) and, based on my findings, most likely *W. carteri*.

Taxonomic and molecular speciation considerations

Although the current literature suggests that *W. carteri* is the only hyriid species in south-western Australia, and the only representative of *Westralunio* in Australia (McMichael &

Hiscock 1958; Walker *et al*. 2001; Walker 2004), I have observed morphological differences in shell shape (unpublished data) in some populations and cryptic speciation could be a possibility, as has been shown in other Australian hyriids (e.g. Baker *et al*. 2003). However, other researchers have shown that shell morphology can vary significantly with varying local environmental conditions even within the same species (e.g. Balla & Walker 1991). Preliminary allozyme analyses from several populations of *W. carteri*, including those with unusually shaped shells, suggested little genetic divergence between populations (M. Adams, South Australian Museum, pers. comm., 2010). Until further molecular analyses are conducted, I neither deny nor confirm the possibility of other hyriid species within south-western Australia, but the currently available published literature suggests only the one species occurs in the region. This is a topic which I did not explore further during the course of my PhD given both time and funding constraints. Nevertheless, changes in taxonomy are important when considering the conservation status of species (e.g. Mace 2004).

2.5 Conclusions

This study has demonstrated the need for robust sets of data to make an accurate conservation assessment using IUCN Red List Criteria. Prior to this study, the lack of the appropriate data and available analyses led to *W. carteri* being moved from Vulnerable to its current Least Concern status. Furthermore, the new data from this study shows further declines of *W. carteri* since its listing as Vulnerable in 1996, with declines expected to continue into the future, which is likely to result in the species status being changed to Endangered (A2c), internationally in the IUCN Red List of Threatened Species, nationally under the *Environment Protection and Biodiversity Conservation Act 1999* and through

Western Australian legislation under the *Wildlife Conservation Act 1950*, all of which utilise the IUCN Red List guidelines. My analysis highlights the importance of referencing adequate high quality data when applying the IUCN criteria. The distribution modelling data show that salinity and perenniality of water were the most important variables in determining the presence or absence of *W. carteri*; the decline of *W. carteri* from its historic range is principally explained by increased salinity; and within perennial sites, turbidity and salinity were most important in determining the likelihood of *W. carteri* presence, but also other variables such as pH, TN and TP have some effect, but are of much lesser importance. Hence, salinity, drying, sedimentation, TN, pH and TP are all threatening processes for *W. carteri*.

Furthermore, salinity tolerance experiments showed that *W. carteri* is acutely intolerant of salinity (LC_{50} = 1.3 – 3.0 g L^{-1}; LC_{95} = 3.2 – 4.3 g L^{-1}), which more or less agreed with the field distribution data because *W. carteri* was absent from systems where mean salinity was greater than 1.5 g L^{-1}. Similar to freshwater fishes of the region (Morrongiello *et al*. 2011), *W. carteri* is generally not found in systems which have historically been non-perennial and is unable to withstand drying for more than a few days when exposed to sunlight without moist sediments or shade, as was shown experimentally.

Knowledge of freshwater mussel life history, including reproductive cycles, host fish requirements, and growth and age estimates (all of which were virtually unknown for *W. carteri* prior to the current study) are important in determining future population viability and conservation management needs for some of the world's most globally threatened fauna (Bogan 1993; Lydeard *et al.* 2004; Strayer 2008). I will address these topics for *W. carteri* in the ensuing chapters.

Chapter 3

Reproductive biology in *Westralunio carteri*

3.1 Introduction

Interspecific and intraspecific comparisons of sexual strategies among the Unionoida are highly variable (Wächtler *et al.* 2001). Many unionids are dioecious and reproduce sexually, although females have been shown to become hermaphroditic in populations with very low densities (Bauer 1987a; Byrne 1998). Oocytes migrate from the ovaries of females into specialized areas in the gills known as marsupia, where they are fertilised and brooded to become embryos which develop into larvae (Bauer & Wächtler 2001; Strayer 2008).

Larval release is often seasonal and opportunistic, particularly in temperate climates, but gametogenesis is generally continuous (Walker *et al*. 2001). In temperate Australia, for example, *Alathyria profuga* (GOULD, 1850) and *Hyridella* spp. brood during the warmer months, but *Cucumerunio novaehollandiae* (GRAY, 1834) broods during autumn and winter (Atkins 1979; Jones *et al.* 1986; Byrne 1998). *Alathyria jacksoni* IREDALE, 1934 and *Velesunio ambiguus* (PHILIPPI, 1847) are seasonal spawners with brooding occurring during winter and early spring and release glochidia in spring-summer (Walker 1981). In tropical environments however, reproduction can occur year-round with multiple broods (Humphrey 1984; Widarto 1996).

Unionoid fecundity is quite large, with broods numbering from a few thousand to several million, depending on the size of glochidia and the adult gravid female (Wächtler *et al*. 2001). Reproductive biology of the Unionidae and Margaritiferidae, most of which occur in the Northern Hemisphere, is well established (Neves & Moyer 1988; Bruenderman & Neves 1993; Downing *et al*. 1992; Downing & Downing, 1993; Kesler & Downing

1997; Jones *et al*. 2004; Schöne *et al*. 2004; Helama *et al*. 2006; Howard & Cuffey 2006; Helama & Valovirta 2008; Haag & Commens-Carson 2008; Haag 2009). Within the Australian Hyriidae, reproductive biology has been studied in seven of the 18 species (Hiscock 1951; Atkins 1979; Walker 1981; Humphrey 1984, 1995; Jones *et al*. 1986; Widarto 1996; Jupiter & Byrne 1998; Byrne 1998). The reproductive biology of *W. carteri*, however, has not been studied previously.

The conservation and aquaculture of the Unionoida is largely dependent on accurate knowledge of reproductive phenology (Kovitvadhi *et al*. 2006; Haag 2009). The purpose of this study was to quantify, for the first time, the timing of gametogenesis, spawning, brooding and glochidia release of *W. carteri*, a crucial step towards understanding the conservation needs for effective management of the species. Due to the temperate nature of the climate in south-western Australia, which exhibits similar seasonal changes as can be found in south-eastern Australia where most reproductive studies of Australasian hyriids have occurred (Walker *et al*. 2001), I hypothesize that reproductive biology in *W. carteri* occurs seasonally.

3.2 Materials and methods

3.2.1 Study area

South-western Australia is characterised by a seasonal climate similar to that of the Mediterranean, with cool wet winters and hot dry summers (Pen 1999). Two populations of *W. carteri* in the region were examined to determine reproductive phenology: one in the Canning River, near Perth and one in the Collie River, near Bunbury (Fig. 3.1). Both sites receive environmental flow releases from reservoirs upstream. Flow releases from the Canning Reservoir maintain the Canning River (Mayer *et al*. 2005). Water flowing into this

dam is typically less than 0.5 g L^{-1} in dissolved salts, rendering it an important source of drinking water (Mayer *et al.* 2005). Water flowing from the Wellington Reservoir maintains the Collie River. The river is considered brackish with mean salinities of 1.1-1.5 g L^{-1} and is typically used for irrigation agriculture (Mayer *et al.* 2005).

3.2.2 Mussel sampling

Samples of *W. carteri* (N = 582, n = 20 from each site on each sampling occasion) were hand-collected collected from the two populations at 4-8 week intervals (from September 2008 to October 2011). Mussels were collected from the same areas within each locality on each sampling occasion. Temperature data were obtained from the Western Australian Department of Water database. The gauging stations nearest to the mussel collection sites were: (1) Canning River –AWRC616027 Seaforth (32°5.53´S, 116°00.63´E) and (2) Collie River –AWRC612043 Rose Road (33°17.95´S, 115°47.96´E) (see Fig. 3.1).

Westralunio carteri were measured with callipers and subsequently anaesthetised in 0.01% benzocaine solution and dissected in the laboratory. Gender was evident by the presence (females) or absence (males) of marsupia on the inner demibranchs of the gills (Wächtler *et al.* 2001), which was confirmed using gonad histology (see Section 3.2.3 below). In females, the marsupium was clearly visible on the inner demibranchs of the gills throughout the year as opaque columns perpendicular to the hinge of the shell.

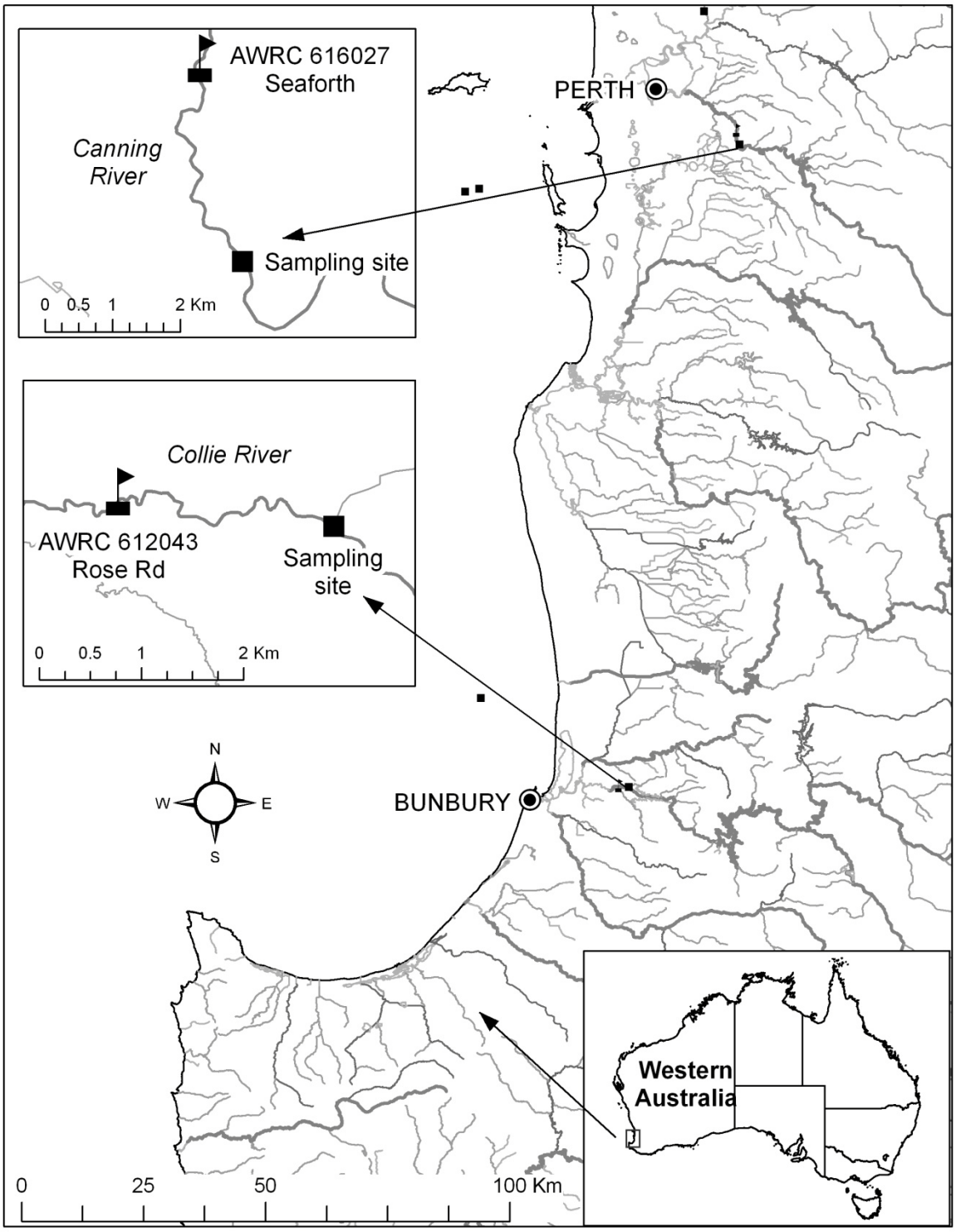

Fig. 3.1 South-western Australia, showing locations of sites sampled for reproductive biology in *Westralunio carteri*. (Spatial data provided by Western Australian Department of Water, under license).

3.2.3 Determination of brooding stage

Gravidity in females was determined by the presence of eggs, embryos or glochidia in the marsupia. Small fractions of the gills from gravid females were excised and larvae were removed and examined so their developmental stage could be determined using light microscopy. The brooding stage of gravid females was separated into four stage classes (Fig. 3.2) based largely on Jones *et al*. (1986) and Byrne (1998): Stage (I) marsupia empty, not swollen; Stage (II) inner demibranchs of marsupia swollen, white in colour from the presence of oocytes or early stage embryos; Stage (III) thickened marsupia, orange to light tan in colour, glochidia not fully formed and shell movements inactive, but circulatory currents within vitelline membrane apparent; Stage (IV) marsupia swollen, brick red in colour; glochidia fully formed; larval teeth present; glochidia active with rapid movements of shells.

3.2.3 Gonad histology

Methods for histological examination of gonads were similar to those reported by Byrne (1998). For gonad histology, a medial portion of the visceral mass, with the foot removed, was dissected from each of the mussels collected during the sampling period and fixed in Bouin's fluid for 24 hours. The fixed viscera were dehydrated in graded ethanols, embedded in paraffin, sliced into 6 μm thick sections, mounted on glass slides, stained with haematoxylin and eosin and glass cover slips were mounted with xylene. Residual viscera were preserved in 100% ethanol for future study. For exceptionally small specimens (<30 mm long), visceral masses were sectioned in levels to ensure mature gonads could be located if they were present.

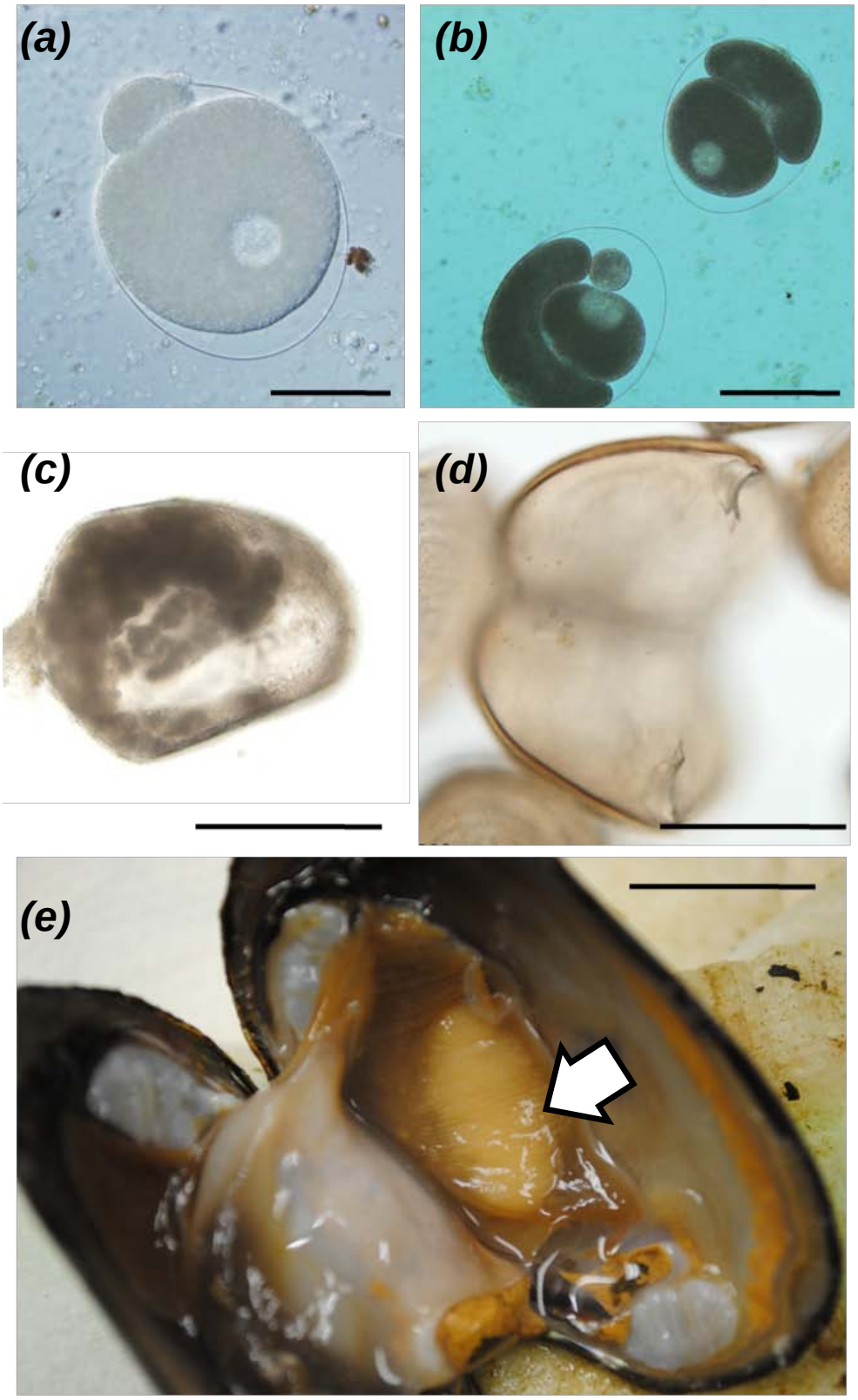

Fig. 3.2 Brooding stages (II-IV) of *Westralunio carteri*. *(a)* Individual eggs or *(b)* early embryos from Stage II marsupia; *(c)* late embryos from Stage III marsupia and *(d)* mature glochidia from Stage IV marsupia of gravid females; scale bars 200 µm. An example of a Stage II female is shown in *(e)* with an arrow indicating the position of the left marsupium; scale bar 20 mm. N.B. Stage I marsupia would be non-gravid and would be lacking the characteristic 'white patch' shown in *(e)*.

Oogenesis from histological sections of the gonads was quantified using methods similar to Jones *et al.* (1986) and Haggerty *et al.* (1995, 2005). Firstly, diameters of 30 oocytes from each female specimen were measured with a ruled cross hair reticule (Fig. 3.3). Only those oocytes which were sectioned through the nucleus were measured. Transects were moved along the X axis of the reticule with the microscope stage being moved right to left. The X and Y diameter of each nucleated oocyte which touched the ruler of the reticule were measured and divided by two for a mean diameter. If 30 nucleated oocytes could not be measured in the first pass, the microscope stage was shifted 10 units (using the ruler) along the Y axis and the process was repeated until all 30 cells were measured. Secondly, the number of oocytes (including those which were not nucleated) was quantified using a tally counter in the first 30 acini within the field of view for each female specimen as the microscope stage was moved in a right to left direction. The total number of oocytes was then divided by 30 for a mean oocyte count for each female.

Using methods from Jones *et al.* (1986) and Haggerty *et al.* (1995, 2005), some preliminary quantification of spermatogenesis from male histological sections of *W. carteri* suggested that male cell types were extremely patchy within acini and different levels of maturity occurred in different regions within the visceral mass. Given these concerns and because published literature suggests that males synchronise with females during spawning (e.g. Jones *et al*. 1986; Haggerty *et al*. 1995, 2005), I did not quantify spermatogenesis. Furthermore, the seasonality of oogenesis and release of glochidia is clearly evident and I assume that males must be in synchrony with females for fertilisation to occur.

All statistical analyses were undertaken using Sigma Plot™ 12.0. Differences in the proportion of males to females between populations were tested for significance by Chi-

square analysis. Temporal effects on oogenesis were analysed by one-way analysis of variance and means were compared using Duncan's multiple range tests.

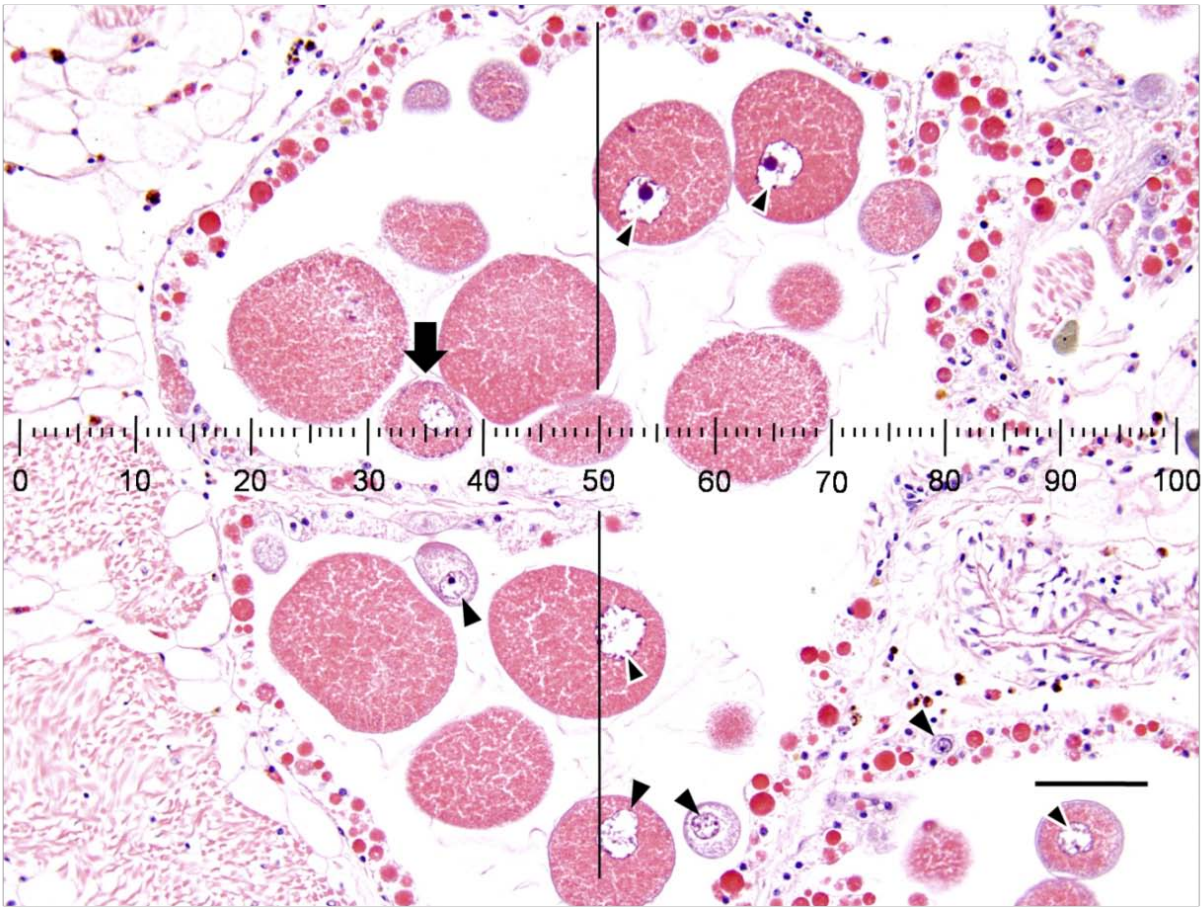

Fig. 3.3 Histological examinations of oocytes in *Westralunio carteri*. Female acinus with oocytes (stained bright pink) at various stages of development. Only those oocytes which contained a visible nuclear envelope (arrows) and touched the graticule ruler (large arrow) were measured. Bar = 100 µm.

3.3 Results

3.3.1 Proportion, maturity and size of each gender

Within the Canning River, males (55.5%) slightly outnumbered females (45.5%), while within the Collie River, females (53.4%) outnumbered males (46.6%); this difference in sex ratio between populations was significant (χ^2 = 6.17, d.f. =1, 791; P < 0.02). No hermaphrodites were observed in the Canning River however, three were found in the Collie River over the duration of the study.

The proportion of each gender in different size classes is presented in Fig. 3.4. Although the proportion of males predominated over females in the 35-39 mm and 40-44 mm size classes within the Collie River, size frequency distributions were similar between males and females for most other size classes and within the Canning River.

The smallest sized *W. carteri* in which gonadal development (i.e. either oogenic or spermatogenic) was apparent from histology was 39.2 and 26.7 mm long for males in the Canning and Collie Rivers, respectively. The smallest sexually mature females measured 27.2 and 45.8 mm long in the Canning and Collie Rivers, respectively. The smallest individuals which did not contain gametes were 31.5 and 25.9 mm in the Canning and Collie Rivers, respectively. Chapter 6 will present age-at-length estimates for these populations.

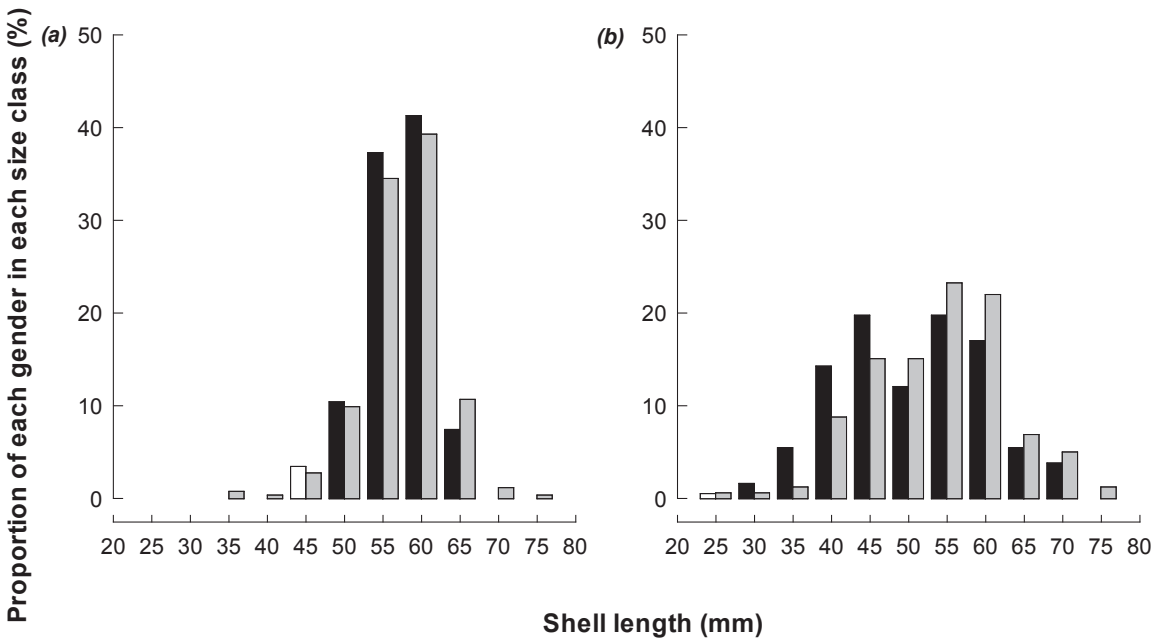

Fig. 3.4 Proportion of males and females of *Westralunio carteri* in relation to shell length within (a) the Canning River and (b) the Collie River. Hermaphrodites and individuals of unknown gender are excluded. Grey bars are males and black bars are females.

3.3.2 Spatial and temporal patterns in reproduction

Oogenesis occurred year-round and the annual cycle was similar between years (Fig. 3.5). There were significant differences in oocyte diameter within the Canning River (F = 10.01; d.f. = 22, 4476; $P < 0.001$) and the Collie River (F = 15.78; d.f. =11, 1758; $P < 0.001$) as well as in the number of oocytes per follicle within the Canning River (F = 3.88; d.f. = 22, 142; $P < 0.001$) and Collie River (F = 2.08; d.f. = 11, 50; $P < 0.04$) among sampling times. Mean oocyte diameter and the mean number of oocytes per follicle were greatest in autumn-winter (May-June), 3-4 months prior to brooding in both rivers sampled for *W. carteri.* Oocyte diameters and counts were smallest during mid- to late summer (January-March) and increased during autumn and early winter (April-June). Oocytes grew from stalks which squeezed away from the wall of the acini to eventually become free-floating within the lumen of the acini, which was also observed in hermaphrodites from the Collie River. A decrease in oocyte size and number within the acini during May-June coincided with the presence of oocytes, or early stage embryos in the marsupia, indicating spawning activity. There was no relationship between shell size (L) and mean number of oocytes per follicle within either the Canning or the Collie Rivers.

Westralunio carteri is a seasonal brooder. Brooding (Stages II-IV) occurred from late winter to early summer in both localities (Fig. 3.6). The proportion of each female brooding stage within marsupia at each sampling time in each locality is shown in Figs. 3.7 and 3.8.

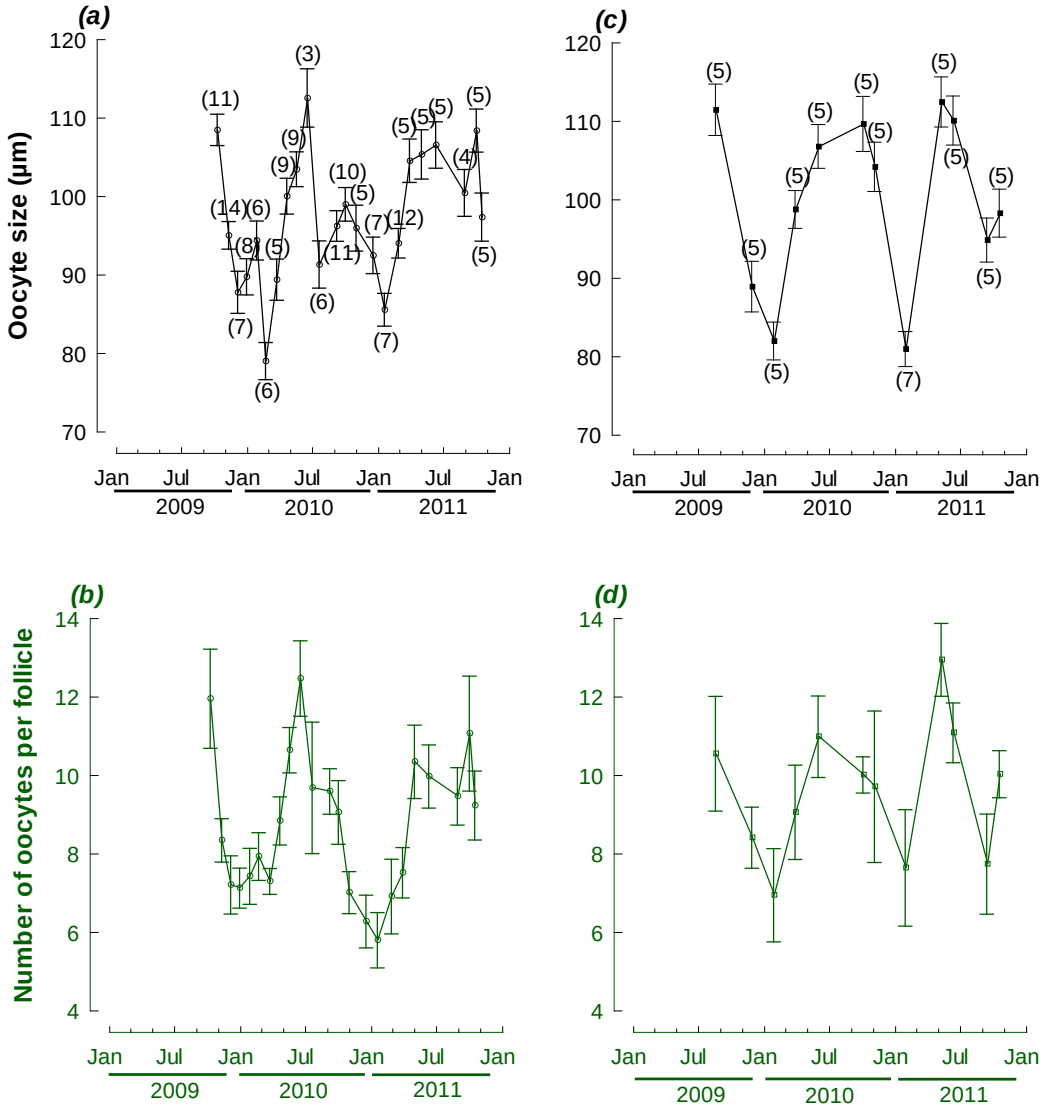

Fig. 3.5 Temporal change in *(a)* mean (± s.e.) oocyte size and *(b)* mean (± s.e.) number of oocytes per follicle in the Canning River and *(c)* mean (±s.e.) oocyte size and *(d)* mean (±s.e.) number of oocytes per follicle in the Collie River from female *Westralunio carteri*. Number of individuals examined is given in brackets in the top row.

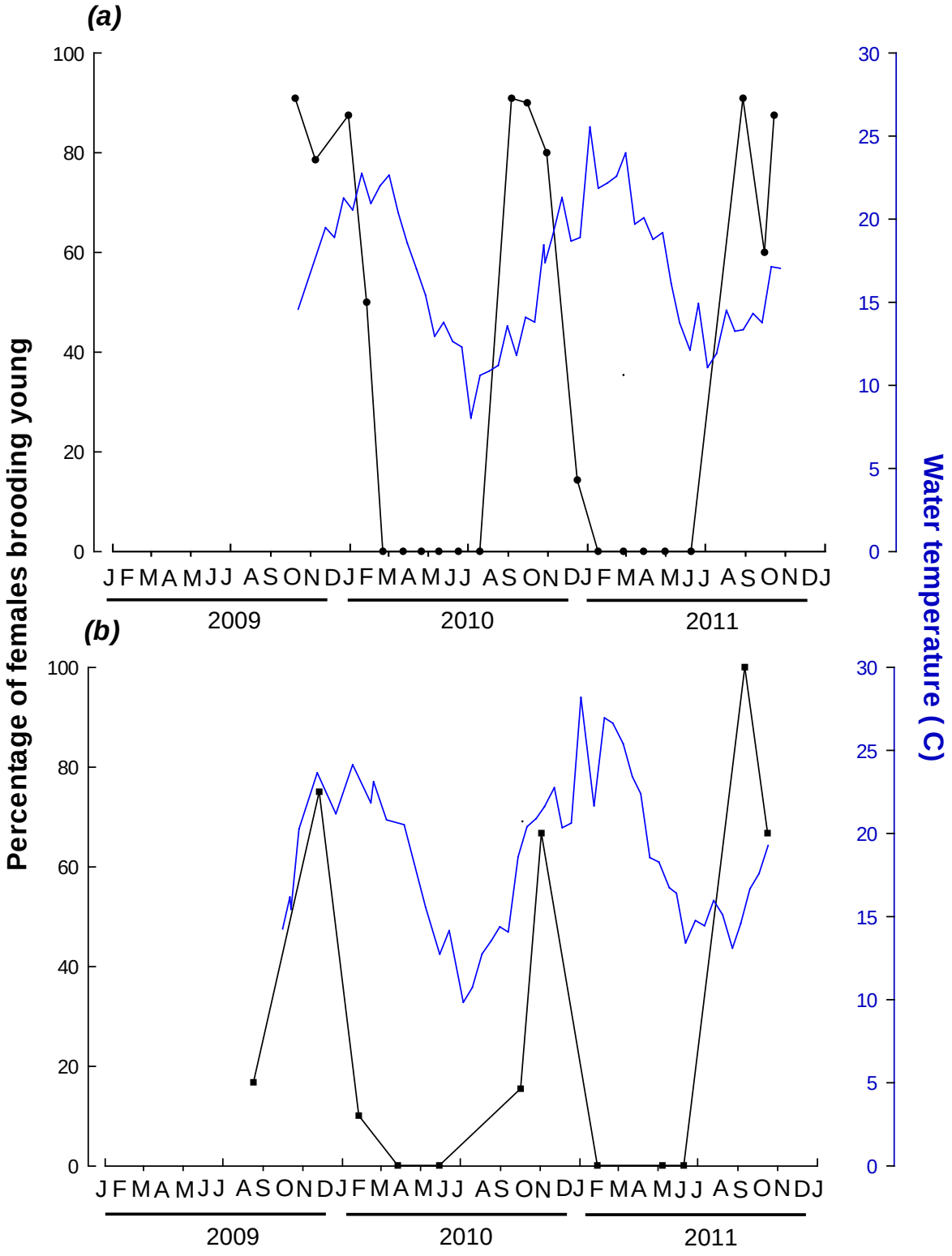

Fig. 3.6 Temporal change in water temperature (blue) and percentage of female *Westralunio carteri* brooding embryos or glochidia in within marsupia (black). *(a)* Canning River; *(b)* Collie River.

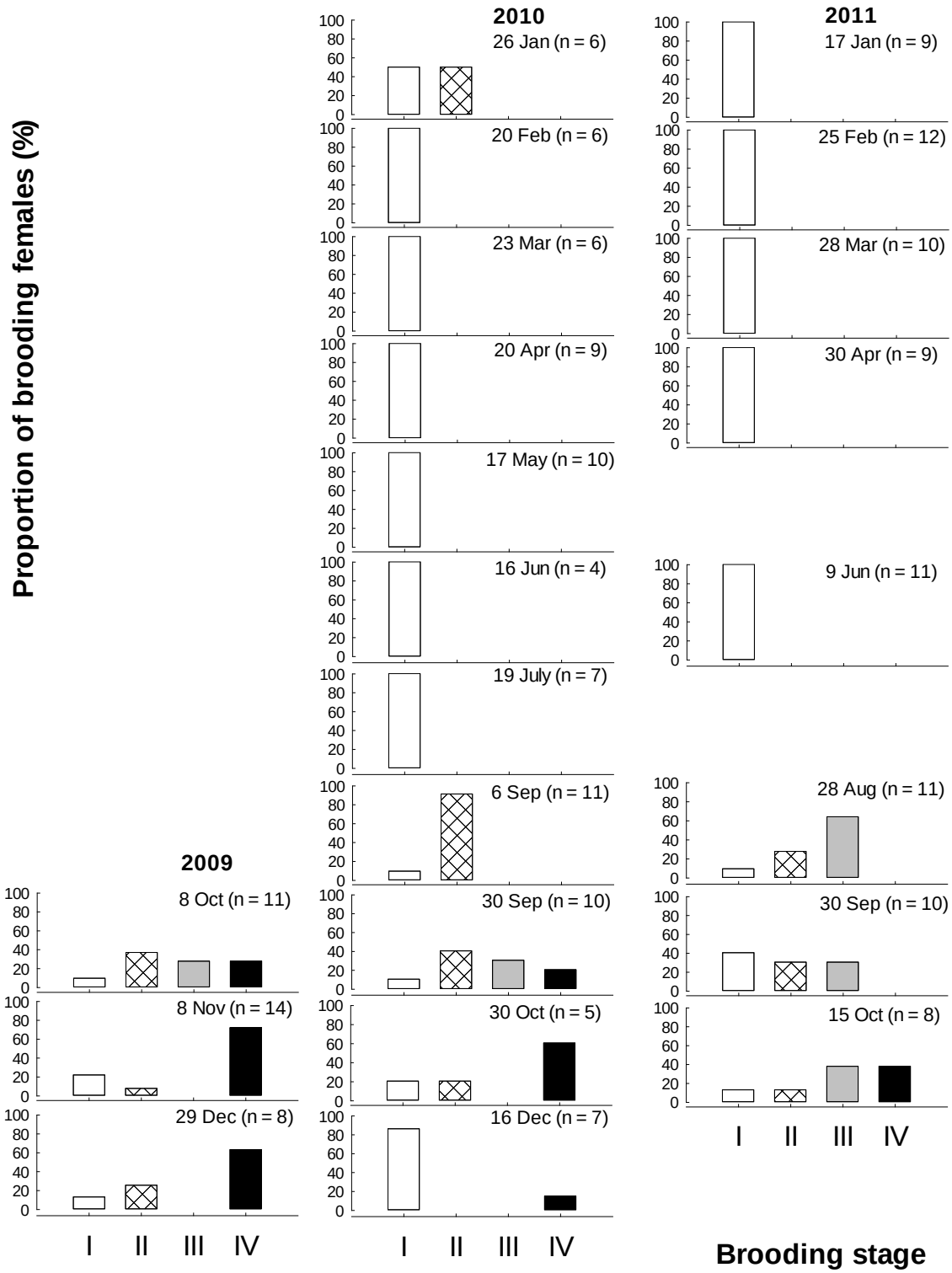

Fig. 3.7 The proportion of each female marsupia brooding stage of *Westralunio carteri* for each sampling period within the Canning River.

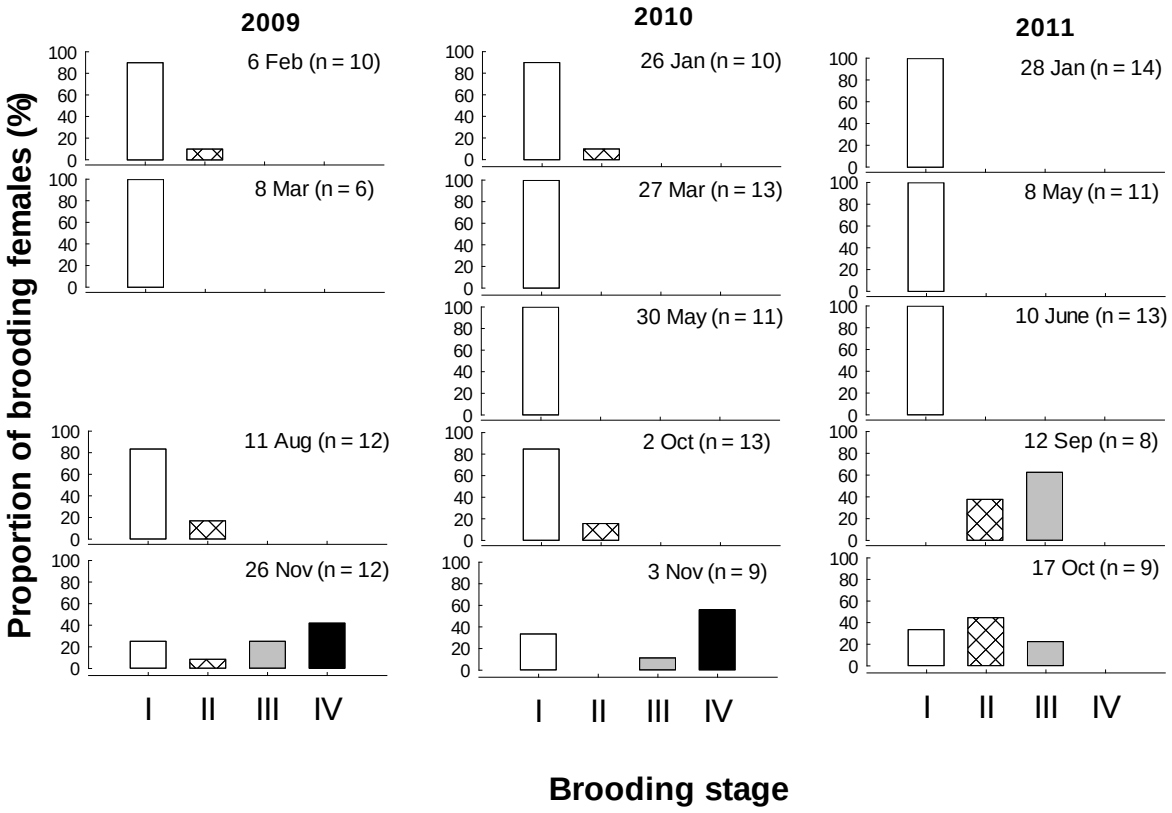

Fig. 3.8 The proportion of each female marsupia brooding stage of *Westralunio carteri* for each sampling period within the Collie River.

3.4 Discussion

This is the first study to elucidate reproductive phenology of *W. carteri*. Within the populations examined, *W. carteri* appeared to have spawned during or shortly after winter water temperatures had begun to warm in August, as oocytes and early stage glochidia (embryos) were first detected in the marsupia of female mussels at this time. Gametogenesis occurred year-round with female gametes present in the gonads for most of the year. However, the production of large oocytes within the gonads peaked in June, again indicating highly synchronous spawning in late winter. Mature glochidia were apparently released between September and December as marsupia were empty from about mid-December to late-July when the cycle was repeated.

Reproduction in *W. carteri* is comparable to other species of tachytictic breeders (brooding in spring and release of glochidia in summer), including *Alathyria jacksoni* IREDALE, 1934 from the Murray-Darling system of south-eastern Australia (Walker 1981), and temperate North American species (Yokely 1972; Matteson 1948; Yeager & Neves 1986; Weaver *et al.* 1991; Bruenderman & Neves 1993; Haggerty *et al.* 1995, 2005). *Hyridella* spp. are longer term, repetitive (bradytictic) breeders which brood successively from October to March and again from April to July in the Macleay River, New South Wales (Jones *et al*. 1986). In *H. depressa* (LAMARCK, 1819) from Lake Burragorang in New South Wales, spawning occurs in July and glochidia are brooded through the warmer months, with marsupia all empty by February-March (Byrne 1998). In *Cucumerunio novaehollandiae* (GRAY, 1834) however, the breeding cycle is opposite, with summer spawning and brooding occurring during autumn and winter (Jones *et al*. 1986). *Velesunio ambiguus* (PHILIPPI, 1847), inhabiting wetlands of the Murray-Darling, is also a seasonal breeder with spawning and brooding during winter-spring and glochidia release in summer, but the species breeds over a longer period than other species and is therefore more adaptable to changing conditions (Walker 1981; Walker *et al*. 2001). Species from tropical Australia, including *V. ambiguus* and *V. angasi* (SOWERBY, 1867) brood throughout the year, producing several clutches of glochidia (Humphrey 1984; Widarto 1993). Generally speaking, glochidiogenesis takes less time in warmer climates of the tropics than it does in more temperate regions, resulting in multiple broods (Ghosh & Ghose 1972; Nagabhushanam & Lohgaonker 1978; Kenmuir 1981; Humphrey 1984; Walker *et al*. 2001).

Oocyte size and count were greatest in spring, a feature common among tachytictic brooders (Yokely 1972; Matteson 1948; Yeager & Neves 1986; Weaver *et al*. 1991;

Bruenderman & Neves 1993; Haggerty *et al.* 1995, 2005). Gametogenesis appears to occur throughout the year in all Australian species which have been studied, and indeed most species elsewhere (Bauer & Wächtler 2001). In seasonal breeders, gonads become packed with advanced gametes (large oocytes) just prior to spawning, leaving little empty space in the lumen of the acini; post-spawning, the gametes are dispersed and less crowded within the acini, but marsupia become packed with oocytes during this time (Byrne 1998).

In south-western Australia, spawning activity and glochidia release in *W. carteri* appears to coincide with temperature change. Water temperature is an important cue for spawning activity in Australian hyriids from temperate climates (Jones *et al.* 1986; Byrne 1998). Atkins (1979) also suggested that temperature change is a cue for glochidia release. In some cases, changes in photoperiod may influence reproductive activity (e.g. Allen 1924; Parker *et al*. 1984). None-the-less, temperature change and changes in stream discharge are more widely accepted as the cue for spawning and glochidia release (Bauer & Wächtler 2001).

Hermaphroditism in *W. carteri* was rare and thus, it is a predominately dioecious species. Byrne (1998) showed that low densities of *H. depressa* led to an increased incidence to hermaphroditism, which may be an adaptation to maintain recruitment. In the northern hemisphere, gender in *Margaritifera margaritifera* LINNEAUS 1758 is density dependent and when population density becomes too low to guarantee fertilization, predominately female populations can function as facultative hermaphrodites. Hermaphroditism can also occur from infection with parasites such as digenetic trematodes which can castrate their host freshwater mussel (Jokela *et al*. 1993; Martel & Trdan 1994; Taskinen *et al*. 1994; Walker *et al*. 2001).

3.5 Conclusions

This study shows that, like freshwater fishes (Prince & Potter 1983; Morrison 1988; Pen & Potter 1990, 1991 a, b; Pen *et al*. 1991; Morgan *et al*. 1995, 2000, 2003; Gill *et al*. 1996; Beatty *et al*. 2010b) and also freshwater crayfish (e.g. Smooth Marron *Cherax cainii* AUSTIN, 2002) (Beatty *et al*. 2003) that spawn during winter and spring in south-western Australia, *W. carteri* is a seasonal tachytictic brooder which spawns annually in mid-winter to early spring (June – August) and releases glochidia in spring to early summer (September to December). Glochidia release coincides with seasonal migrations of some freshwater fishes of south-western Australia (see Beatty *et al*. 2010b). The importance of this relationship and synchrony between glochidia release and host fish movement will be discussed in Chapter 5.

Chapter 4

Morphological and morphometrical description of the glochidia of *Westralunio carteri*

Klunzinger, M.W., Beatty, S.J., Morgan, D.L., Thomson, G.J. & Lymbery, A.J. (2012). Description of glochidia and manner of their discharge from *Westralunio carteri* Iredale, 1934 (Bivalvia: Unionoida: Hyriidae). *Molluscan Research*

4.1 Introduction

Glochidia morphology is variable within and between the various taxonomic groups (Surber 1912; Wächtler *et al*. 2001). The last systematic review of the Australasian Hyriidae (McMichael &Hiscock 1958) was based primarily on the shell and anatomy of adults. This classification is uncertain, however, because taxa beneath the family level are defined by morphological features which vary in response to local environmental conditions (Balla & Walker 1991; Walker *et al*. 2001; Baker *et al*. 2004), soft anatomy lacks differentiation and morphological data of glochidia are incomplete (Walker 2004; Ponder & Bayer 2004). Although descriptions of the glochidia of the Australasian Hyriidae are fragmentary, diversity in larval tooth morphology, shell shape and shell dimensions suggest that they may have systematic value (Walker *et al*. 2001).

Of the 30 nominal species in nine genera of Australasian Hyriidae, glochidia have been described for 11 species (Percival 1931; Parodiz & Bonetto 1963; Walker 1981; Humphrey 1984; Jones *et al*. 1986; Widarto 1993; Jupiter & Byrne 1997; Walker *et al*. 2001). Glochidia release strategies have received a lot of attention in many Northern Hemisphere species (e.g. Kat 1984; Kraemer & Swanson 1985; Haag *et al*. 1995), but little is known about the mechanism of release in the Australasian Hyriidae (but see Atkins 1979; Jones *et al.* 1986; Jupiter & Byrne 1997). Walker *et al.* (2001) suggest that glochidial morphology may have taxonomic potential in the Australasian Hyriidae, noting that

reproductive characters and glochidial morphology have long been used in South American hyriid systematics (see Pimpão *et al.* 2012).

Despite having been accepted as a unique taxon for nearly 80 years, the glochidia of *W. carteri* have never been described. In this chapter, I describe glochidial morphology and shell dimensions and illustrate the release of glochidia by *W. carteri* for the first time. My hypothesis is that *W. carteri* has a distinct morphology and shell dimensions which are different from other Hyriidae (e.g. Jones *et al.* 1986), but the mechanism for their release will be similar to other Australasian Hyriidae which release glochidia in strands of mucus (e.g. Atkins 1979).

4.2 Materials and methods

Gravid female *W. carteri* (*n* = 3 each) were collected from two populations, separated by a distance of approximately 200 km: one in the Canning River (32°07′S; 116°01′E) and the other from the Collie River (33°38′S; 115°91′E). Live mussels were hand-collected and transported to the laboratory in plastic buckets containing river water. Samples were maintained in the buckets at room temperature, observed for glochidia release and subsequently dissected. Gravidity was apparent macroscopically from the appearance of thickened, orange to reddish coloured marsupia in the inner two thirds of the inner demibranchs of the females' gills (Walker 1981; Jones *et al*. 1986; Byrne 1998). Released glochidia were removed from the floor of the bucket, placed on a glass slide using a glass pipette and examined under a light microscope for behaviour. A sub-sample of live glochidia were removed for scanning electron microscopy (SEM) and gills containing mature glochidia were preserved in 100% ethanol for later analysis. A total of 120 preserved mature glochidia (*n* = 20 from each of three females within each locality) were

flushed from the females' gills with distilled water and placed on a glass slide. Intact mature glochidia, free of their vitelline membrane, were measured to the nearest 10 µm for length, height and hinge length (Fig. 4.1) using a graticule ruler within the eyepiece of a Motic BA-210 light microscope. For SEM, live glochidia were fixed for 2h in gluteraldehyde, dehydrated in graded ethanols, placed on a glass cover slip lined with double-sided sticky tape attached to a specimen stub, critical point dried, sputter-coated with gold, and examined and photographed with a Philips XL 20 SEM.

Given that terminology used to describe glochidia characteristics varies in the literature, some definitions of terms are required here. 'Glochidial' or 'larval tooth' is the distal projection proximal to the ventral apex to either valve projecting inward or more-or-less perpendicular to the lateral plane of either valve of the glochidia (Atkins 1979; Mansur & Silva 1999; Morgan *et al*. 2011; Klunzinger 2011; Klunzinger *et al*. 2011a; 2012b). 'Cusps' are sharp terminations of the glochidial teeth (Mansur & Silva 1999; Pimpão *et al*. 2012). A 'protuberance' refers to a rounded projection originating from the base of the glochidial/larval tooth (Walker 1981; Vale *et al*. 2005; Pimpão *et al*. 2012). The 'larval thread' is a strand of tissue inserted between the hinge of the valves which unravels and remains attached to the vitelline membrane upon glochidial 'hatching' (Matteson 1948; Hiscock 1951; Atkins 1979; Jones *et al*. 1986; Jupiter & Byrne 1997).

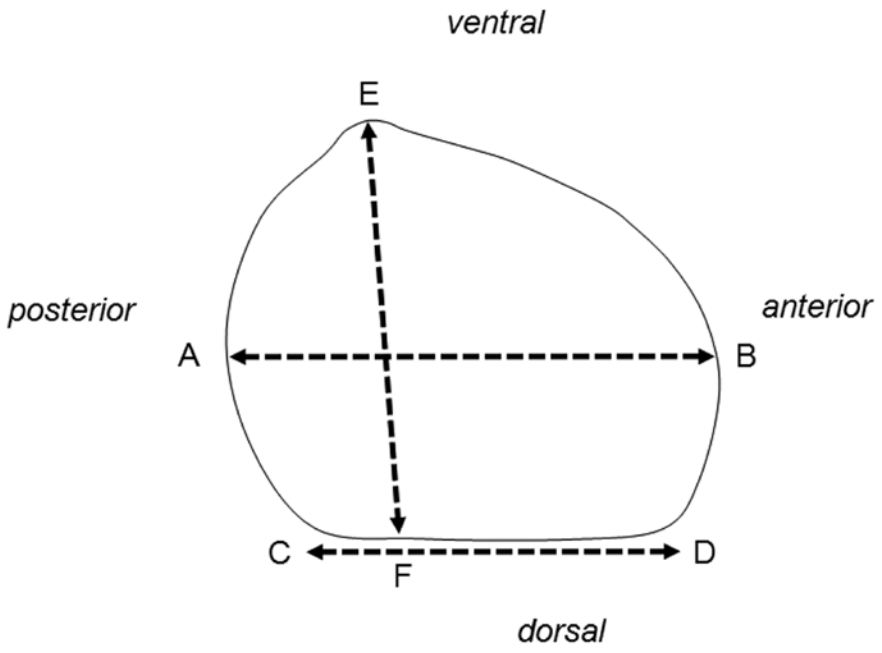

Fig. 4.1 Morphometric measurements of glochidia shells (right valve): A-B = Length; C-D = Hinge length; E-F = Height (derived from Jones *et al.* 1986). Anatomical orientation descriptors are labeled according to Hoggarth (1987).

4.3 Results

Female *W. carteri* released thick strands of mucus that contained glochidia enveloped in vitelline membranes. Upon release, the mucus strands began to thin, liberating mature glochidia still within their vitelline membranes. Shortly thereafter, glochidia excised themselves by expanding their valves, rupturing their vitelline membranes (Fig. 4.2). The larval thread then unraveled, still attached to the vitelline membrane and the soft tissues of the glochidia (Fig. 4.2). At that point glochidia began to characteristically 'wink' with rapid contractions of their valves, indicating maturity. The larval thread was extremely sticky, as was the vitelline membrane.

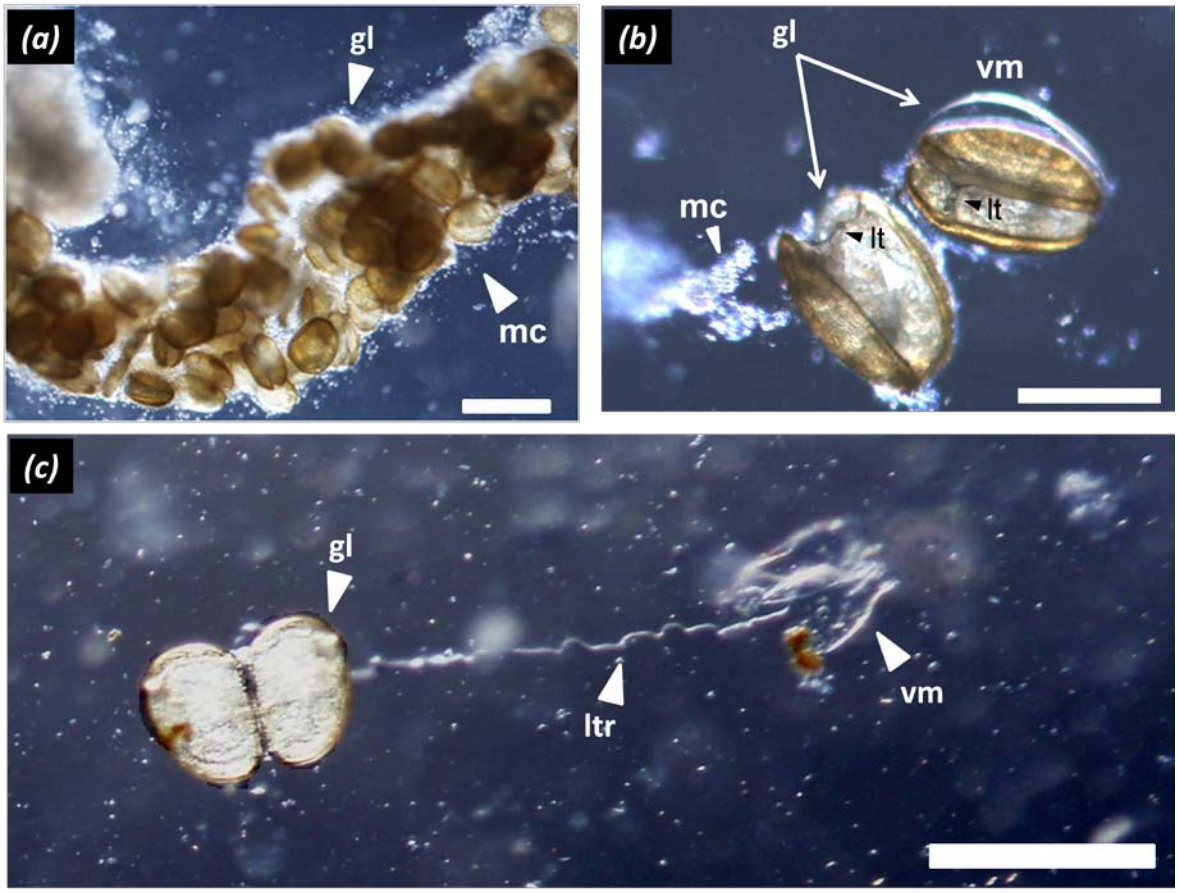

Fig. 4.2 Light microscopy of: *(a)* glochidia (gl) packaged in a mucus strand (mc) released from the exhalant siphon of a gravid adult *Westralunio carteri*, scale bar: 500 µm; *(b)* individual glochidia floating free of the mucus strand, but remain within the vitelline membrane (vm), larval teeth (lt) shown, scale bar: 200 µm; *(c)* an individual glochidia which has expanded its valves to rupture the vitelline membrane (vm), but remains attached via the larval thread (ltr): this individual was actively 'winking' after hatching, scale bar: 500 µm.

Glochidial shell morphology of *W. carteri* is illustrated in Fig. 4.3. The valves were operated by a single adductor muscle attached to the medial portion of each valve. The shells of *W. carteri* were subtriangular and inequilateral in shape with a smooth porous surface which lacked surface spikes. The apex of the ventral edge was off-centre and closest to the posterior region of the glochidial shell. The teeth were more or less interlocking when shells of glochidia were closed. Teeth were slightly curved towards the adductor muscle with a concave protuberance on the base of the right valve tooth and a convex protuberance on the base of the left valve tooth. The glochidial tooth of the right valve was 'spear-like', terminating with three sharp cusps and the tooth of the left valve

was blunt with two rounded cusps with a groove at the midpoint to accommodate the middle cusp of the right valve tooth. Morphometric measurements and morphology of glochidia shells of *W. carteri* and other Australasian Hyriidae are presented in Table 4.1.

Table 4.1 Comparison of glochidia dimensions in the Australasian Hyriidae. Values presented are means with (±) standard errors.

Species	N	Mean length (μm)	Mean Height (μm)	Hinge length (μm)	Ht/Lth (%)	Hinge /Lth (%)	Reference
Alathyria jacksoni	10	272 ±2.88	253 ±2.91	192 ±2.88	93	70.6	Walker (1981)
Alathyria profuga	20	239 ±1.12	204 ±0.45	165 ±0.89	85	69	Jones *et al.* (1986)
Cucumerunio novaehollandiae	50	52.2 ±0.08	64.1 ±0.03	35	116	64	"
Echyridella menziesii	-	360	280	-	78	-	Percival (1931)
	-	323	277	-	86	-	McMichael & Hiscock (1958)
Hyridella australis	50	73.9 ±0.07	94.7 ±0.04	40	128	68	Jones *et al.* (1986)
Hyridella depressa	50	253 ±0.71	244 ±0.71	152 ±0.85	97	60	"
	5	243 ±5.38	249 ±1.79	-	102	-	Jupiter & Byrne (1997)
Hyridella drapeta	-	330	230	-	71	-	Atkins (1979)
Velesunio ambiguus	10	247 ±2.97	210 ±3.89	173 ±2.94	85	70	Walker (1981)
	-	250	220	-	88	-	McMichael & Hiscock (1958)
	15	263 ±1.78	232.6 ±2.40	200.3 ±1.27	88.4	76.2	Widarto (1993)
Velesunio angasi[1]	-	289	267	222	92	77	Humphrey (1984)
Westralunio carteri							
(Canning River)	**60**	**306 ±0.90**	**252 ±0.83**	**213 ±1.01**	**82.3**	**69.4**	**This study**
(Collie River)	**60**	**309 ±1.37**	**250 ±1.20**	**211 ±1.20**	**80.9**	**68.1**	**This study**

[1] Measurements estimated from photomicrograph in Humphrey (1984)

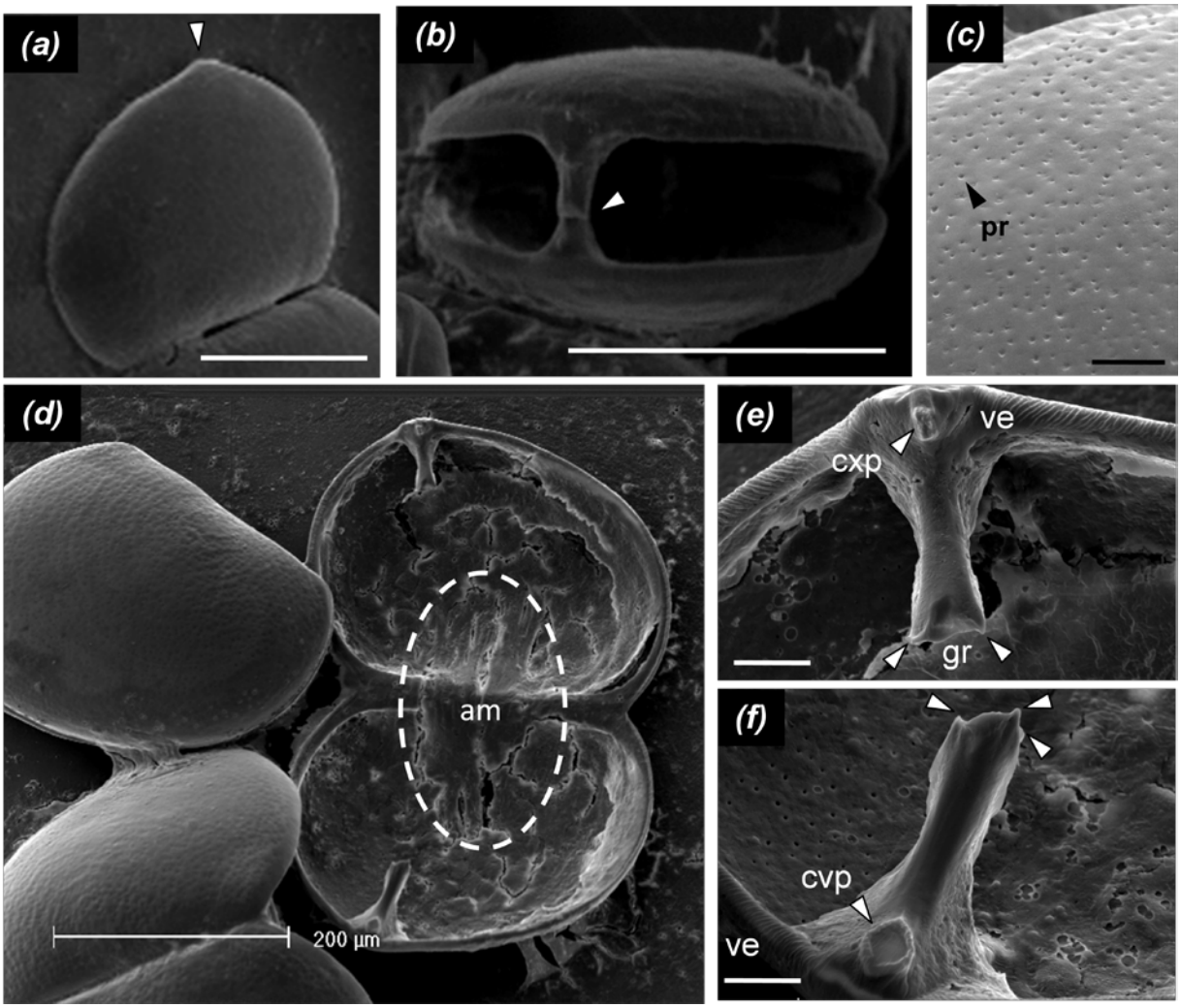

Fig. 4.3 Scanning electron microscopy of glochidia of *Westralunio carteri*. *(a)* Shell shape inequalateral and subtriangular with ventral edge apex (arrow) located off-centre and closest to the anterior end of a left valve. Scale bar: 200 µm. *(b)* Closed shell, glochidial teeth interlocking (arrow). Scale bar: 200 µm. *(c)* Detail of shell surface: smooth with pores (pr) and lacking surface spikes. Scale bar: 20 µm. *(d)* Ventral view, valves open, anterior end on left; adductor muscle (am). *(e)* Larval tooth protruding from ventral edge (ve) of right valve; two blunt cusps (arrows) with centrally located groove (gr) to accommodate the insertion of the tooth from the right valve; protuberance convex (cxp). Scale bar: 20 µm. *(f)* Glochidial tooth protruding from ventral edge (ve) inward on left valve: three cusps apparent (arrows); protuberance concave (cvp). Scale bar: 20 µm.

4.4 Discussion

The glochidium of *W. carteri* is morphologically and morphometrically distinct from other species of Hyriidae. The species lacks shell surface spikes (unlike some Amazonian hyriids described by Pimpão *et al.* 2012), has singular teeth which have a unique morphology and the shells are of a distinct size. In *Velesunio* and *Alathyria* spp., the teeth terminate to a single point (Walker 1981; Humphrey 1984; Widarto 1993); in most *Hyridella*, *Diplodon*, *Cucumerunio* and *Echyridella* spp., the terminal portion of the larval teeth is bifurcated, ending in two points (see Percival 1931; Jones *et al.* 1986; Jupiter & Byrne 1997; Pimpão *et al.* 2012). Like *Velesunio* and *Alathyria* spp., *W. carteri* has a larval thread whereas *Hyridella* spp. apparently lack a larval thread (Jones *et al.* 1986; Jupiter & Byrne 1997).

Pimpão *et al.* (2012) were able to identify and distinguish among genera and species and classify supra specific taxa of Amazonian Hyriidae by canonical discriminant analysis of glochidial shell dimensions and shape. Glochidial dimensions and shape differ markedly among some Australasian species but not others (e.g. among *H. depressa* and *H. drapeta* in Jones *et al.* 1986 and Jupiter & Byrne 1997). Shell size and shape has the potential to differentiate among different glochidial species parasitising fish (e.g. Wiles 1975), which may be especially useful in regions where several species of glochidia co-occur (e.g. Kennedy & Haag 2005). Although this is not particularly relevant in south-western Australia, where *W. carteri* is the only freshwater mussel species, in other regions of Australia there may be multiple species of hyriids which co-occur and recognising the various hyriid glochidia when they are attached to fish could be determined using size measurements as suggested in Jones *et al.* (1986). From an ecological standpoint, the ability to recognise various species of glochidia becomes particularly crucial in systems where adult freshwater mussel diversity is high. For example, Kennedy & Haag (2005)

were able to classify 72 to 79% of total glochidia examined in a North American River which contained 21 species of unionids. These researchers also found that intraspecific variation in glochidia size was low for all but one species, suggesting that glochidia size may be useful for differentiating species.

From a functional perspective, smaller glochidia such as those of *Margaritifera margaritifera* (LINNAEUS, 1758) tend to attach to the gills of host fishes, whereas larger glochidia with larval teeth are found primarily on the fins of their hosts (Bauer 1994). Bauer (1994) also suggests that larger glochidia with shorter host retention times tend to be host generalists, utilising many host fish species, whereas the smaller glochidia are retained for a longer period, have notable growth while on their host and tend to be host-specific. *Westralunio carteri* is a host generalist, attaches almost exclusively to the fins of host fishes (Klunzinger *et al.* 2012b) and has one of the largest glochidia of the Australasian species which have been studied.

Strategies for presenting glochidia for attachment to host fishes are diverse in the Unionoida (Bauer & Wächtler 2001). Within the Australian Hyriidae, glochidia have been reported being released in masses of jelly (Walker 1981) or in worm-like mucus strings which resemble fish food (Jones *et al.* 1986; Jupiter & Byrne 1997). In South American Hyriidae, glochidia are released in mucus, but some species present glochidia in mucus strings while others release glochidia in mucus clusters (Lima & Avelar 2010). In *W. carteri*, glochidia during this study were released in tan coloured mucus strands. The mucus strands and the very sticky larval threads may assist glochidia by suspending them from aquatic vegetation or other structures in a ‘cobweb-like’ arrangement, thus improving their chances of contacting host fishes foraging amongst them (Matteson 1948; Klunzinger *et al.* 2012b).

4.5 Conclusions

The present study showed that the glochidia morphology and shell measurements of *W. carteri* are distinct from other Australasian Hyriidae and although the larval teeth are not bifurcated as in the Hyridellini reported by Jones *et al*. (1986), they are morphologically different from other Velesunioninae which have been studied (Walker 1981; Humphrey 1984; Widarto 1993). The size of *W. carteri* (306-309 µm long) and subtriangular shape will render them recognizable on host fishes (see Chapter 5). The method of their release from females is similar to other Australasian species (Atkins 1979; Walker 1981) and the larval threads may assist in the attachment to the fins of host fishes (Klunzinger *et al*. 2012b). The presence or absence of a larval thread, the morphology of larval teeth and the size of glochidial shells appear to be important in distinguishing some species of Australasian Hyriidae, but more descriptions of the various glochidia of the other 20 species may prove useful in the systematics of the regional group.

Chapter 5

Glochidia ecology in wild fish populations and laboratory determination of competent host fishes for *Westralunio carteri*

Klunzinger, M.W., Beatty, S.J., Morgan, D.L., Thomson, G.J. & Lymbery, A.J. (2012). Glochidia ecology in wild fish populations and laboratory determination of competent host fishes for an endemic freshwater mussel of south-western Australia. *Australian Journal of Zoology* **60,** 26-36.

5.1 Introduction

Factors controlling successful attachment and metamorphosis of freshwater mussels to the juvenile stage are complex and multi-faceted. They include environmental factors, such as water temperature, water depth and habitat composition, adult mussel density and host factors, such as behaviour, seasonal migrations, endemic distributions, abundance, and immune response (Arey 1932b; Bauer & Vogel 1987; Rogers & Dimock 2003; Dodd *et al*. 2005; Strayer 2008).

Few studies have quantified glochidia ecology in wild populations of fishes in Australia or other regions in the Southern Hemisphere (Walker *et al*. 2001). Although a number of Northern Hemisphere studies have identified host fishes in a laboratory setting, few have examined glochidia ecology in wild populations (Strayer 2008).

The aim of this study was to determine the host fish species of the glochidial stage of *W. carteri*, quantify the prevalence and intensity of glochidia infestation throughout its range, determine which fish species may be competent hosts to sustain the life-cycle of the mussel and discuss factors that may influence the distribution and abundance of glochidia in wild fish populations. Based on previous studies of the host fishes for glochidia of other Australasian Hyriidae, I hypothesise that *W. carteri* is a host generalist, having multiple host fishes, but that alien fishes will be less suitable as hosts than native fishes.

5.2 Materials and methods

5.2.1 Field sampling of host fishes

In total, 1005 fishes from 11 species (four alien and seven native) were captured from 18 sites (Fig. 5.1) using two-winged fyke nets (11.2 m wide, 0.8 m deep, 2mm nylon mesh), seine nets (3.0 m wide, 1.0 m deep, 2 mm nylon mesh) and/or electro-fishing (Smith-Root® Model LR20) during November and December 2010. All fishes were identified to species (Morgan *et al*., 1998, 2011) and measured for total length (*TL*) to the nearest 1 millimetre.

For *Tandanus bostocki* WHITLEY, 1944 (*n* = 306), glochidia were easily identified in the field from whitish, bladder-like cysts on the surface of the fish (Klunzinger *et al*. 2011a). A sub-sample (*n* = 5) of infested *T. bostocki* and other fishes (*n* = 699) were transported live to the laboratory, where they were anaesthetised in AQUI-S™ and examined for glochidia using a dissecting microscope. The fins, body, gill filaments, opercula, eyes and mouths were examined for glochidia and their location was recorded.

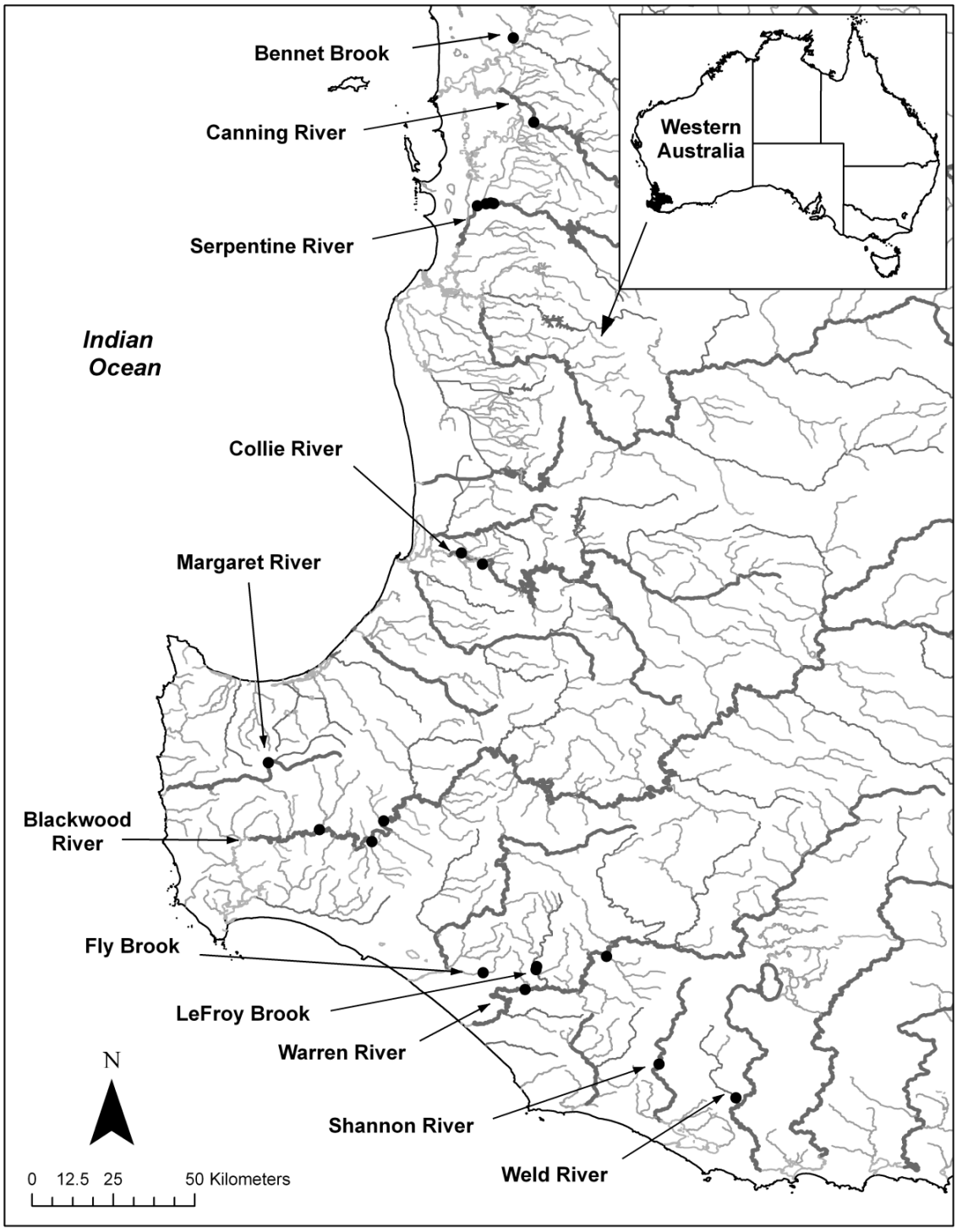

Fig. 5.1 The South West Coast Drainage Division of Western Australia, showing the locations of sampling sites for fishes examined for glochidia of *Westralunio carteri*. (Spatial data provided by Western Australian Department of Water, under license).

5.2.2 Glochidia exposure trials

To determine whether glochidia of *W. carteri* metamorphose to the juvenile stage on different hosts, during 19 September to 6 November 2011 seven species of fish (*Afurcagobius suppositus* (SAUVAGE, 1880), *Carassius auratus* LINNAEUS, 1758, *Geophagus brasiliensis* (QUOY & GAIMARD, 1824), *Gambusia holbrooki* (GIRARD, 1859), *Nannoperca vittata* (CASTELNAU, 1873), *Pseudogobius olorum* (SAUVAGE, 1880) and *T. bostocki*) were exposed to gravid female *W. carteri* in the laboratory. *Afurcagobius suppositus*, *Gam. holbrooki* and *T. bostocki* were sourced from the Collie River; *C. auratus* and *N. vittata* were purchased from Veba's Aquarium Supply (O' Connor, Western Australia 6163); *Ps. olorum* and *G. brasiliensis* were sourced from Bennett Brook. Fishes were captured using fyke nets or seine nets, transported to the laboratory and maintained in aquaria with continuous bio-filtration and aeration several weeks prior to the exposure trial.

A sample (n = 10) of adult *W. carteri* were hand-collected from each of the 18 field study sites and identified as *W. carteri* based on McMichael & Hiscock (1958) and Walker (2004). Mussels were examined, macroscopically for gravidity by holding valves open and manipulating the foot and visceral mass so that marsupia could be scrutinised for mature glochidia. Maturity was determined by the presence of swollen red marsupia, similar to Stage IV Hyriidae elsewhere (Jones *et al*. 1986; Byrne 1998; see also Chapter 3). For comparison, two gravid adult *W. carteri* from the Collie River site were anaesthetized in 0.01% benzocaine solution and dissected. Glochidia were removed and examined under a compound microscope or dehydrated in graded ethanols, placed on a glass cover slip attached to a specimen stub, critical point dried, sputter-coated with gold, and photographed in a Philips XL 20 Scanning Electron Microscope (SEM).

Mussels, which were observed to have released mature glochidia, were exposed to fishes for two hours in continuously aerated 9 L plastic buckets which contained dechlorinated tap water. Glochidia maturity was evident by the examination of a mucus sample which contained glochidia actively 'blinking' and free of their vitelline membrane. Each bucket contained 10 individual fishes of a particular species and 20 gravid individual mussels. Exposed fishes were transferred to individual plastic fish hatcheries (Resun® Model FH-01, Guangzhou, China) and mounted inside 45L aquaria containing continuously aerated sponge bio-filters and dechlorinated tap water. Fishes were fed a diet of bloodworms daily.

Commencing on the second day post-exposure, individual fishes were captured by hand and placed on 75 mm diameter round blank agar plates containing enough water to cover the gills of each fish, and the fins quickly (< 1 min) examined for glochidia under a dissecting microscope. Infested fishes were maintained in the isolation chambers and uninfested fishes were maintained in separate aquaria. Prevalence data for the laboratory trials were not recorded because the aim of the experiment was to determine whether glochidia metamorphose to the juvenile stage, rather than a quantified analysis of infestivity. The water from the bottom of each isolation chamber was siphoned and transferred into another agar plate and scrutinised for the presence of either glochidia or juvenile *W. carteri* microscopically. Immediately following examination, fishes were transferred back into the chambers and placed into their respective aquaria. This process was repeated every second day post-exposure until glochidia were no longer found attached to the fins of infested fishes and juveniles appeared in the isolation chambers.

5.2.3 Statistical analysis

For each fish species at each locality, and for each fish species over all localities, I calculated mean glochidia prevalence (percentage of fish infested) and intensity (number of glochidia per infested fish). Ninety five percent confidence limits were calculated for prevalence, assuming a binomial distribution and intensity, from 2,000 bootstrap replications, using Quantitative Parasitology 3.0 (Rozsa *et al*. 2000). Differences in prevalence among fish species or sites and the tissue site of infestation among species were tested by Chi-square analysis and differences in intensity by a non-parametric Kruskal-Wallis test. The effect of *TL* on prevalence was tested by comparing the *TL* of infested and uninfested fish using a Mann-Whitney U-test and the relationship between *TL* and intensity was tested by Spearman's correlation analysis. A Bonferroni adjustment was made for multiple comparisons of prevalence and intensity, to ensure an experiment-wide error rate of 5%.

5.3 Results

5.3.1 Prevalence and intensity of glochidia on fish hosts

In wild systems, glochidia were attached to and encysted on 10 of the 11 fish species examined, with mean prevalences over all sites ranging from 0 to 90.5% and mean intensities from 1 to 6 glochidia per infested fish (Table 5.1).

Table 5.1 Overall mean glochidia prevalence and intensity in 11 different fish species from the South West Coast Drainage Division of Western Australia. Mean values for prevalence and intensity of glochidia are shown in bold; 95% confidence limits (calculated from 2,000 bootstrap replications) are shown in parentheses. Reported values were calculated from the overall mean of the mean glochidia prevalences and intensities for each fish species within each sampling site. Fish species are listed in order of greatest to least glochidia prevalence.

Family	**Species**	**No. of individuals examined**	**Mean Prevalence (%)**	**Mean Intensity (No. glochidia per infested fish)**
Gobiidae	Swan River goby, *Pseudogobius olorum* (SAUVAGE, 1880)	45	**90.5** (75.9-96.3)	**3.7** (0.0-9.6)
Gobiidae	South-western goby, *Afurcagobius suppositus* (SAUVAGE, 1880)	20	**75.0** (58.5-96.4)	**2.3** (1.3-3.2)
Galaxiidae	Western minnow, *Galaxias occidentalis* OGILBY, 1899	135	**62.6** (50.4-67.6)	**2.7** (1.3-4.0)
Percichthyidae	Western pygmy perch, *Nannoperca vittata* (CASTELNAU, 1873)	191	**44.8** (39.4-53.9)	**2.4** (1.2-3.6)
Poeciliidae	Eastern gambusia, *Gambusia holbrooki* (GIRARD, 1859)[A]	114	**41.0** (30.4-49.0)	**1.7** (1.1-2.3)
Percichthyidae	Nightfish, *Bostockia porosa* CASTELNAU, 1873	76	**29.1** (4.8-30.2)	**3.9** (0.0-8.4)
Atherinidae	Western hardyhead, *Leptatherina wallacei* (PRINCE, IVANTSOFF & POTTER, 1982)	32	**21.9** (9.3-39.9)	**1.1** (0.8-1.5)
Poeciliidae	One-spot livebearer, *Phallaceros caudimaculatus* (HENSEL, 1868)[A]	43	**18.6** (8.3-33.4)	**1.5** (0.9-2.1)
Plotosidae	Freshwater cobbler, *Tandanus bostocki* WHITLEY, 1944	306	**9.2** (6.1-12.6)	**2.5** (0.0-8.2)
Cyprinidae	Goldfish, *Carassius auratus* LINNAEUS, 1758[A]	19	**5.3** (0.0-26.0)	**6.0**[B] ---
Cichlidae	Pearl cichlid, *Geophagus brasiliensis* (QUOY & GAIMARD, 1824)[A]	24	**0.0** ---	---
Total		1005		

[A]Alien fish species

[B]Reported intensity is based on one single infested fish; 95% C.I. could not be calculated; Dashes indicate intensity and 95% C.I. could not be determined because no fishes were infested.

Because different fish species were captured at different sites, Table 5.1 confounds differences in glochidiosis among species with differences among sites. In seven sites where two or more species of fish were collected in sufficient numbers (i.e. n ≥ 10 fish), there were significant differences in prevalence among species in two sites and significant differences in intensity among species in one site (Table 5.2). In five sites, both native and alien fishes were captured and there were no consistent differences in prevalence or intensity among these groups (Table 5.2). For six species which were sampled from two or more sampling sites in sufficient numbers (i.e. n ≥ 10 fish), there were significant differences in prevalence among sites for four species, and significant differences in intensity among sites for two species (Table 5.3).

There were no significant differences in the *TL* of fish infested and uninfested with glochidia for most species (Fig. 5.2; Table 5.4). However, infested *T. bostocki* were significantly larger than uninfested *T. bostocki* ($n = 306$, $U = 1799.0$, $P < 0.001$), as were *B. porosa* ($n = 76$, $U = 373.5$, $P = 0.04$) and a significant positive relationship was found between *TL* and glochidia intensity in *T. bostocki* ($n = 28$, Spearman's correlation, $P < 0.0001$). No relationships between *TL* and glochidia intensities were observed in any other fishes. Infested *Gam. holbrooki* tended to be larger in *TL* than uninfested *Gam. holbrooki*, appearing as a trend ($n = 114$, $U = 1229.0$, $P = 0.09$). Generally, infestations occurred most often on the fins, but occasionally on other tissues (Table 5.5) and there were significant differences among species; fins were most heavily infested ($\chi^2 = 235.7$, d.f. = 54, $P < 0.001$).

Table 5.2 The number of fishes of each species where 10 or more fish were collected within each river and examined for glochidia of *Westralunio carteri*, the proportion of each species infested by glochidia and the mean intensity (number of glochidia per infested fish) of each species within each river**.** Confidence intervals (95%) are shown in parentheses. Comparisons were made between species within rivers; differences in prevalence were analysed by Chi-square and differences in intensity by Mann-Whitney or Kruskal-Wallis tests. Test statistics in bold with an asterisk indicate significance at the $P<0.05$ level, with the Bonferroni correction.

River Name (site)	Fish Species	N	Mean Prevalence (%)	Prevalence χ^2 (d.f.)	Mean Intensity	Intensity Statistic (d.f.)
Bennett Brook	*Gal. occidentalis*	21	100.0 (83.9-100.0)		5.9 (5.0-7.2)	
	Ps. olorum	17	94.1 (71.3-99.9)	**26.7 (3)***	5.6 (3.8-8.5)	*H* = 6.6 (2)
	N. vittata	17	35.3 (14.2-61.7)		2.7 (1.0-7.0)	
	Geo. brasiliensis[A]	24	0.0 (0.0-14.25)		-	
Canning River	*N. vittata*	15	26.7 (7.8-55.1)		1.8 (1.0-2.5)	
	Ph. caudimaculatus[A]	43	18.6 (8.4-33.4)	3.3 (2)	1.5 (1.0-2.0)	*U* = 13.5 (1)
	Gal. occidentalis	11	0.0 (0.0-28.5)		-	
Serpentine River						
1 (Bush Forever site 368)	*Ps. olorum*	25	88.0 (68.8-97.5)		4.4 (3.1-6.4)	
	Gal. occidentalis	31	83.9 (66.3-94.55)	7.5 (2)	4.5 (3.1-7.0)	***H* = 10.0 (2)***
	Gam. holbrooki[A]	48	62.5 (47.4-76.1)		2.0 (1.5-2.6)	
2 (Lowlands Gauging Station)	*Gal. occidentalis*	18	16.7 (3.6-41.4)	0.2 (1)	1.0[B]	*U* = 6.0 (1)
	Gam. holbrooki[A]	34	11.8 (3.3-27.5)		1.0[B]	
Collie River	*A. suppositus*	18	83.3 (58.6-96.4)		2.3 (1.5-3.2)	
	N. vittata	16	37.5 (15.2-64.6)		2.2 (1.0-3.3)	
	Gam. holbrooki[A]	18	27.8 (9.7-53.5)	**109.5 (4)***	1.6 (1.0-2.2)	*H* = 3.3 (4)
	L. wallacei	32	21.9 (9.3-40.0)		1.1 (1.0-1.3)	
	T. bostocki	222	2.3 (0.7-5.2)		1.4 (1.0-1.6)	
Margaret River	*N. vittata*	12	100.0 (73.5-100.0)	3.5 (1)	7.1 (5.1-9.2)	*U* = 11.0 (1)
	Gal. occidentalis	20	75.0 (50.9-91.4)		1.5 (1.1-2.0)	
	Gal. occidentalis	14	50.0 (23.0-77.0)		1.9 (1.1-2.3)	
Shannon River	*B. porosa*	25	32.0 (14.9-53.5)	1.5 (2)	4.1 (2.4-6.3)	*H* = 4.0 (2)
	N. vittata	13	30.8 (9.1-61.4)		1.5 (1.0-1.8)	

[A]Alien fish species
[B]95% C. I. could not be calculated

Table 5.3 The number of fishes collected for each species within each river where 10 or more individuals were captured and examined for glochidia of *Westralunio carteri*, the proportion of each species infested by glochidia and the mean intensity (number of glochidia per infested fish) of each species. Confidence intervals (95%) are shown in parentheses. Comparisons were made between rivers within species; differences in prevalence were analysed by Chi-square and differences in intensity by Mann-Whitney or Kruskal-Wallis tests. Test statistics in bold with an asterisk indicate significance at the $P<0.05$ level, with the Bonferroni correction.

Fish Species	River Name (Site no.)	No. fish examined	Mean Prevalence (%)	Prevalence χ^2 (d.f.)	Mean Intensity	Intensity Statistic (d.f.)
B. porosa	Serpentine River 2	17	35.3 (14.2–61.7)	0.1 (2)	2.3 (1.3-3.0)	H = 14.5 (2)
	Shannon River	25	32.0 (14.9-53.5)		4.1 (2.5-6.5)	
	Serpentine River 1	48	64.0 (49.2-77.1)		2.0 (1.6-2.6)	
Gam. holbrooki[A]	Collie River	18	27.8 (9.7-53.5)	**24.4 (1)***	1.6 (1.0-2.2)	U = 3.0 (1)
	Serpentine River 2	34	11.8 (3.3-27.5)		1.0[B]	
Gal. occidentalis	Bennett Brook	21	100.0 (83.9-100.0)		5.9 (4.9-7.3)	
	Serpentine River 1	31	85.7 (69.7-95.2)		1.9 (3.1-6.4)	
	Margaret River	20	75.0 (50.9-91.4)	**58.2 (4)***	1.5 (1.1-1.9)	***H* = 31.0 (3)***
	Serpentine River 2	18	16.7 (3.6-41.4)		1.0[B]	
	Canning River	11	0.0 (0.0-28.5)		---	
N. vittata	Margaret River	12	100.0 (73.5-100.0)		7.1 (5.1-9.1)	
	Fly Brook	40	42.5 (27.0-59.1)		2.1 (1.5-3.4)	
	Lefroy Brook	40	42.5 (27.0-59.1)		1.8 (1.2-3.1)	
	Collie River	16	37.5 (15.2-64.6)	**21.5 (6)***	2.2 (1.0-3.3)	***H* = 22.2 (6)***
	Bennett Brook	17	35.3 (14.2-61.7)		2.7 (1.0-5.8)	
	Shannon River	13	30.8 (9.1-61.4)		1.5 (1.0-1.8)	
	Canning River	15	26.7 (7.8-55.1)		1.8 (1.0-2.5)	
Ps. olorum	Bennett Brook	17	94.1 (71.3-99.9)	0.4 (1)	5.6 (3.7-8.8)	U = 152.0 (1)
	Serpentine River 1	25	88.0 (68.8-97.5)		4.4 (3.1-6.4)	
T. bostocki	Blackwood River	84	27.4 (18.2-38.2)	**46.3 (1)***	4.2 (2.3-8.6)	U = 46.5 (1)
	Collie River	222	2.3 (0.7-5.2)		1.4 (1.0-1.6)	

[A]Alien fish species
[B]95% C. I. could not be calculated

Table 5.4 Comparison of median total lengths (*TL*) of fishes infested and uninfested with glochidia of *Westralunio carteri*. Differences are significant at the $P<0.05$ level and trends appear at the $P<0.10$ level, given in bold. Species are listed in alphabetical order.

Species	***TL* infested (mm)**	***TL* uninfested (mm)**	***U*-test**	***P*-value**
A. suppositus	57.0	61.0	30.0	0.84
B. porosa	**92.0**	**80.0**	**373.5**	**0.04**
C. auratus†	120.0	75.0	4.5	0.50
Gal. occidentalis	64.5	75.0	1914.5	0.52
Gam. holbrooki†	**40.0**	**35.0**	**1229.0**	**0.09**
Geo. brasiliensis†	---	69.0	---	---
L. wallacei	54.0	51.0	73.5	0.54
N. vittata	43.0	43.0	4356.0	0.63
Ph. caudimaculatus†	37.5	31.0	99.5	0.21
Ps. olorum	37.0	42.0	64.5	0.21
T. bostocki	**272.0**	**145.0**	**1799.0**	**<0.001**

†Alien fish species

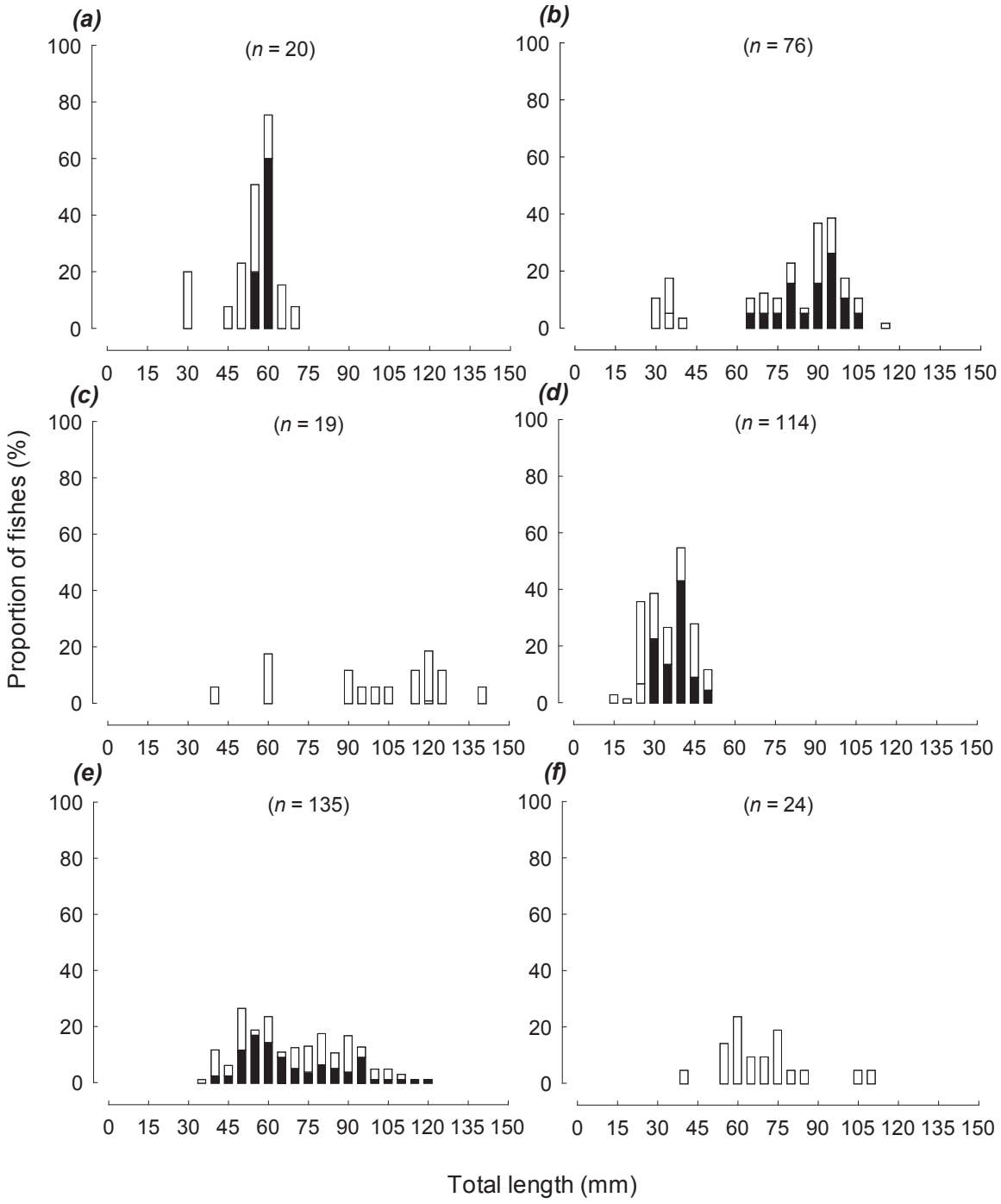

Fig. 5.2.1 Length-frequency histograms of fish species infested (black bars) and uninfested (white bars) with glochidia of *Westralunio carteri*: *(a) Afurcagobius suppositus*; *(b) Bostockia porosa*; *(c) Carassius auratus*; *(d) Galaxias occidentalis*; *(e) Gambusia holbrooki*; *(f) Geophagus brasiliensis*.

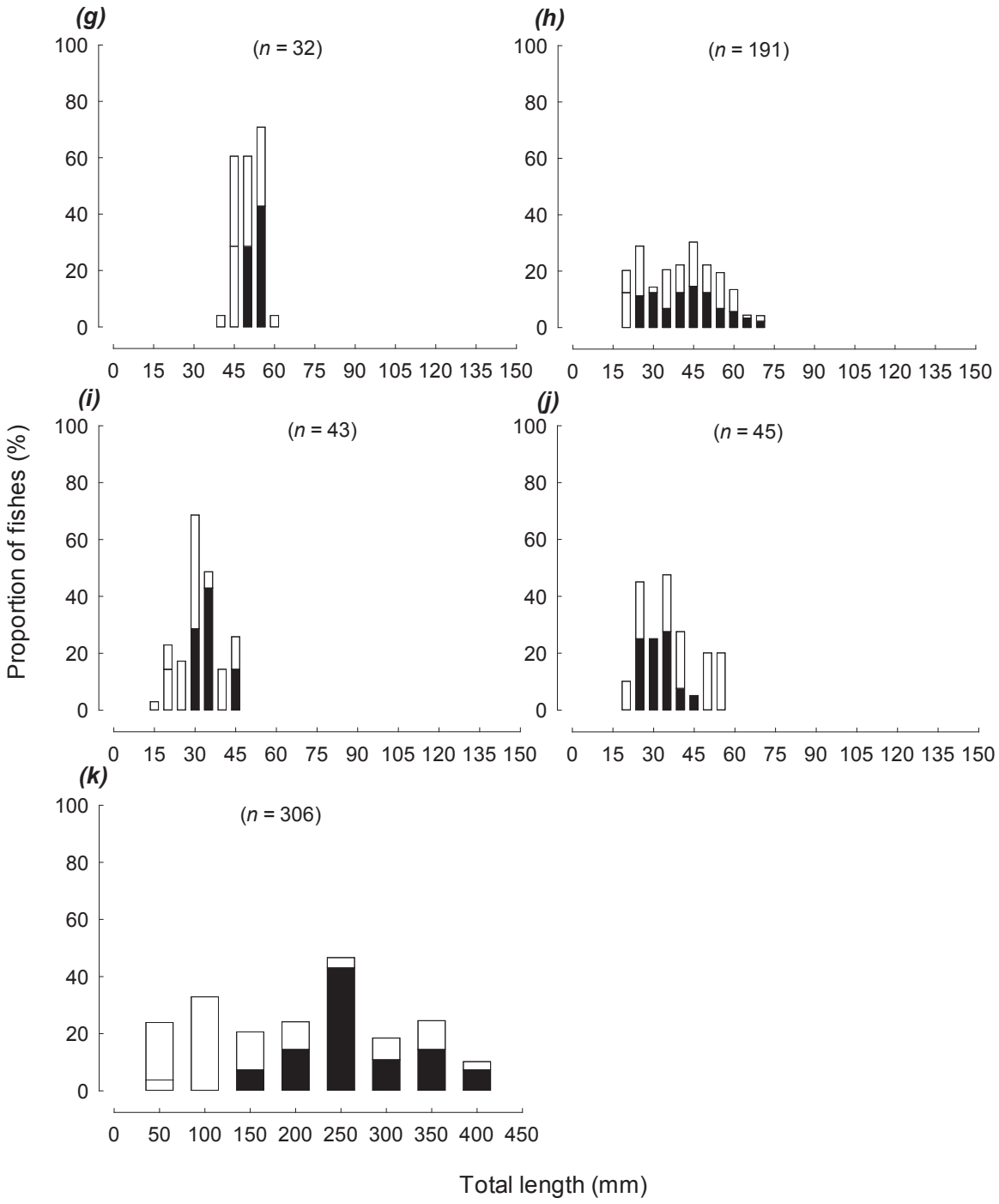

Fig. 5.2.2 Length-frequency histograms of fish species infested (black bars) and uninfested (white bars) with glochidia of *Westralunio carteri*: *(g) Leptatherina wallacei*; *(h) Nannoperca vittata*; *(i) Phalloceros caudimaculatus*; *(j) Pseudogobius olorum*; *(k) Tandanus bostocki*.

Table 5.5 The number of fish with glochidia in each infestation site within each species. Species and infestation sites are significantly related (χ^2 = 235.728, d.f. = 54, P < 0.001). *Carassius auratus*, *L. wallacei* and *Ph. caudimaculatus* were excluded from statistical analysis due to a low number of infested individuals (n < 10), but shown here for comparative purposes. ×, anatomical feature not present.

		Infestation site											
Species	**No. of infested individuals**	**eye**	**mouth**	**opercula**	**gills**	**body**	**pectoral fins**	**pelvic fins**	**anal fins**	**dorso-caudal fin**	**1st dorsal fin**	**2nd dorsal fin**	**caudal fin**
A. suppositus	15	---	---	---	---	---	6	6	4	×	1	---	5
B. porosa	19	---	---	---	---	---	14	7	4	×	8	×	10
C. auratus[A]	1	---	---	---	---	---	---	---	---	×	---	×	1
Gal. occidentalis	80	---	6	4	---	---	50	26	22	×	36	×	19
Geo. holbrooki[A]	45	1	---	---	---	---	2	3	14	×	13	×	28
L. wallacei	7	---	---	---	---	---	4	2	2	×	---	×	---
N. vittata	89	1	2	4	---	2	25	19	32	×	43	16	29
Ps. olorum	40	---	---	2	---	---	32	14	10	×	11	13	11
Ph. caudimaculatus[A]	8	---	---	---	1	---	1	3	1	×	5	×	×
T. bostocki[B]	28	---	---	1	1	---	1	---	1	22	2	×	×

[A]Alien fish species

[B]A subsample of five *T. bostocki* was examined for glochidia on all tissues in the laboratory, but 23 fishes were examined for glochidia on fins only in the field.

5.3.2 Glochidia metamorphosis

Glochidia were released on tan-coloured mucus strings from the exhalent siphons of *W. carteri*. Glochidia were visible through translucent host tissue as brown, sub-triangular shaped shells attached to the fins of infested fish, similar to those obtained from the gill marsupia of adult female *W. carteri* (Fig. 5.3), with the exception of *T. bostocki*, in which cysts were not translucent, but contained glochidia.

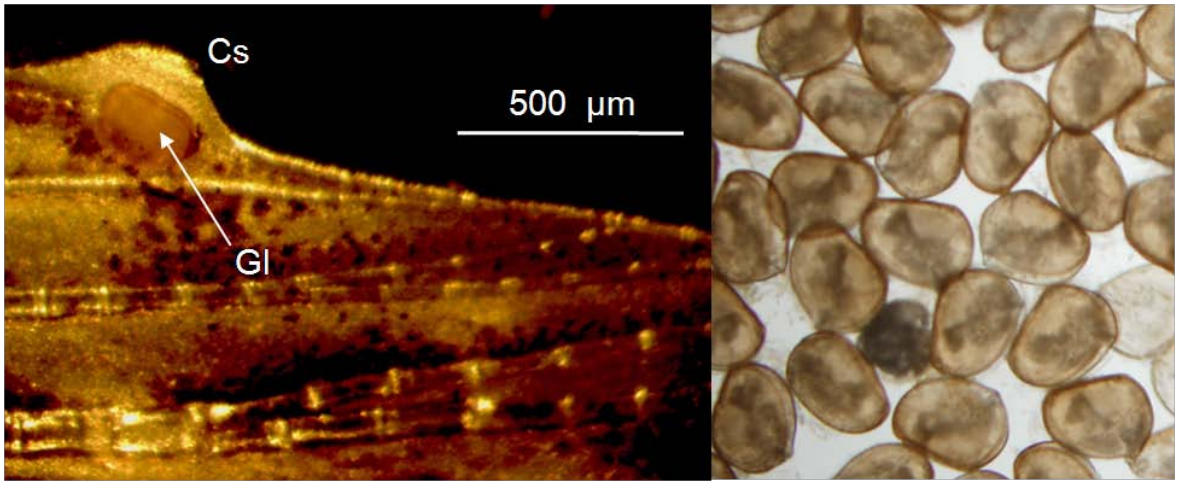

Fig. 5.3 (left) Glochidia (Gl) attached to and encysted (Cs) on the dorsal fin of a Swan River goby, *Pseudogobius olorum*. (right) Free glochidia obtained from the marsupia of an adult freshwater mussel, *Westralunio carteri.*

Two species of native euryhaline fishes (*A. suppositus* and *Ps. olorum*), two species of native freshwater fishes (*N. vittata* and *T. bostocki*) and one alien fish species (*Gam. holbrooki*) were found to be competent hosts for glochidia of *W. carteri*. Glochidia were encysted on these fishes and underwent metamorphosis to the juvenile mussel stage (Fig. 5.4) in the laboratory. Although attachment may have occurred briefly, glochidia were not encapsulated on two other alien fish species (*C. auratus* and *Geo. brasiliensis*). Time to metamorphosis ranged from 20 to 27 days (Table 5.6).

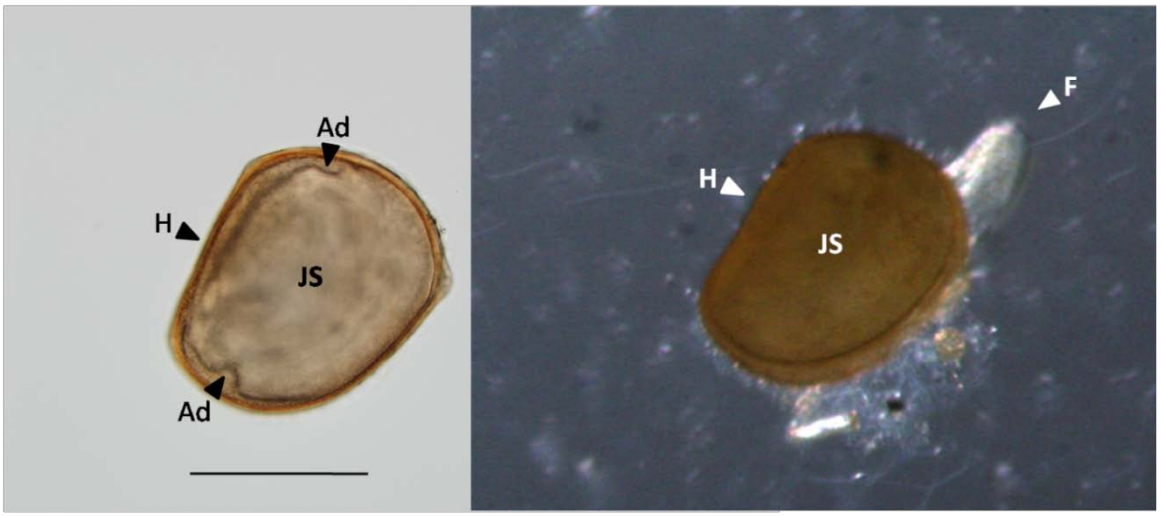

Fig. 5.4 Juvenile *Westralunio carteri* two days after detachment from a *Pseudogobius olorum*. F, foot; H, hinge ligament; JS, juvenile shell; Ad, rudimentary adductor muscles. Bar = 250 µm.

Table 5.6 Metamorphosis from the glochidia to the juvenile stage of *Westralunio carteri* exposed to potential host fish species under controlled laboratory conditions. Rows are arranged alphabetically by fish species. Dashes indicate metamorphosis was not observed.

Fish species	**No. individuals exposed**	**Time to metamorphosis (d)**
Afurcagobius suppositus [B]	17	20
Carassius auratus[A]	26	---
Gambusia holbrooki[A]	15	21
Geophagus brasiliensis[A]	5	---
Nannoperca vittata [C]	20	21-27
Pseudogobius olorum [B]	10	26
Tandanus bostocki[C]	18	21

[A]Alien species
[B]Native euryhaline species
[C]Native freshwater species.

5.4 Discussion

This study suggests that *W. carteri* is a host generalist for native fishes (*A. suppositus*, *B. porosa*, *Gal. occidentalis*, *L. wallacei*, *N. vittata*, *Ps. olorum* and *T. bostocki*), although *B. porosa*, *Gal. occidentalis* and *L. wallacei* need to be tested to confirm whether they are competent host species. *W. carteri* does not appear to utilise the alien *C. auratus* or *Geo. brasiliensis*. However, the alien *Gam. holbrooki* was confirmed as a competent host. The alien *Ph. caudimaculatus* may be a potential host based on field studies, but I did not test the species in the laboratory; its distribution in Western Australia is very restricted (Morgan *et al*. 2004). Other hyriids are also native endemic host generalists (Bonetto & Ezcurra 1963; Humphrey 1984; Widarto 1996; Walker *et al*. 2001).

I found differences in glochidia prevalence among different host species, both overall and in comparisons of different species within localities. Whether this was due to differences in contact rate or in host compatibility is unclear, but it is probably a combination of both factors (see Strayer 2008). Contact rate may be influenced by site-specific factors, such as water depth and adult mussel density (Strayer 2008). Although these were not measured in the current study, I did find significant differences in glochidia prevalence on the same (four of six) species of fish in different localities, which suggests that environmental factors influenced contact rate between glochidia of *W. carteri* and its hosts, although larger and more uniform sample sizes would help solidify statistical arguments. Contact rate is also likely to be influenced by host-specific factors, such as habitat utilisation; fishes more closely associated with mussel beds and the sediments may have an increased likelihood of encountering glochidia (Humphrey 1984; Widarto 1996; Strayer 2008). In the current study, prevalence of glochidia infestation was greatest on the two native euryhaline goby species. These are both dense-bodied, negatively buoyant

benthic-feeders with modified pelvic fins for clinging to the benthos (Pen *et al*. 1992; Pusey & Bradshaw 1996; Morgan *et al*. 1998, 2011; Allen *et al*. 2002).

The method of glochidia release in *W. carteri* however, may facilitate contact with hosts other than strictly benthic species. The mucus strings had active glochidia attached to them and *W. carteri* has been observed expelling material from its exhalent siphon up to a distance of 5 cm vertically (Klunzinger 2011). This illustrates the potential of the species to distribute glochidia onto other surfaces in a 'cob-web-like' manner as described by Matteson (1948) and could also target fishes that feed and occupy mid-water and at least very shallow surface areas of streams. Moreover, mid-water species, such as *N. vittata* tend to occupy shallower habitats near sloping river banks, as do *W. carteri*, based on my observations.

Contact rate is unlikely to provide a complete explanation of differences in prevalence of glochidia infestation among host species. *Tandanus bostocki*, which is also a species closely associated with the benthic regions of streams, had only a low to moderate prevalence of infestation. This species also appeared to have reacted to attached glochidia, encapsulated within a much thicker cyst that contained 'pus-like' material, a feature which was absent in other host species. Studies on other Unionoida show evidence of innate and adaptive immune responses from fish hosts (Bauer and Vogel 1987; Rogers & Dimock 2003; Dodd *et al*. 2005), but I cannot substantiate any claims of immune response in *T. bostocki* or other host species without immunological study.

The effect of host size was evident in *T. bostocki* and *B. porosa* in which infested fishes were larger in *TL* than uninfested fishes. These are two of the largest of the region's 11 native freshwater fishes and have a wide range in *TL* (Morgan *et al*. 1998, 2011). Although introduced fishes such as *G. brasiliensis* and *C. auratus* are deep bodied and can

grow to relatively sizeable lengths (≥ 200 mm), none larger than 140 mm were examined in this study and from the almost complete lack of glochidiosis in these fishes, no inference on size can be made. In the case of *Gam. holbrooki*, larger fish tended to become infested more so than their smaller counterparts, although the trend was not significant. Bauer & Vogel (1987) support the idea that larger fish have a larger fin area and filter more water through their gills, resulting in a greater chance of glochidia infestation, a concept which supports our observations for *T. bostocki*, *B. porosa* and *Gam. holbrooki*. Other authors (e.g. Blažek & Gelnar 2006) also reported a positive relationship with host size on glochidia infestation elsewhere. On the contrary, smaller and younger fish sometimes carry greater glochidia loads than older and larger fish, which could be attributed to immune naivety (Young & Williams 1984; Klunzinger *et al*. 2010).

Although previous studies have shown that attachment and metamorphosis of glochidia is more successful on native than on alien fishes (Bauer 1987a-c; Rogers *et al*. 2001; Wächtler *et al*. 2001), successful transformation has sometimes been observed on alien host fishes (Hiscock 1951; Atkins 1979; Walker 1981; Widarto 1996; Watters 1997; Watters & O'Dee 1998; Strayer 2008). The alien *Gam. holbrooki* had a moderately high prevalence of infestation in the field and exhibited successful metamorphosis in the laboratory, a finding also observed for other hyriids of eastern Australia (Walker 1981; H. Jones pers. comm. 2010). Infested *G. brasiliensis* were never found either in the field or in laboratory trials, although only 20 fish were examined. Although six glochidia were found on a single *C. auratus* in the field, it is uncertain whether they were viable and successful attachment and metamorphosis did not occur in the laboratory. Furthermore, wild *C. auratus* examined in this study appeared to be undergoing fin fragmentation, possibly as a result of glochidia attachment, an observation supported by Rogers-Lowery & Dimock

(2006), who found that resistant fish slough epithelial cells in response to glochidia. Laboratory trials (Hiscock 1951; Walker 1981; Widarto 1996) have previously suggested that *C. auratus* and carp (*Cyprinus carpio* LINNAEUS, 1758) are unsuitable hosts for Australian Hyriidae, possibly because the mucus produced by their epithelial tissues is too thick to allow glochidial attachment, even though hyriid glochidia can be induced to attach in a laboratory setting, the glochidia are usually shed within 2-3 hours (Walker 1981).

The time required for transformation of glochidia to the juvenile stage is largely dependent on temperature (Walker 1981; Humphrey 1984; Hastie & Young 2003). In this study, I placed temperature data loggers in a few aquaria to estimate whether transformation time of *W. carteri* glochidia might be affected by temperature during the trial period. In some cases, different species of fishes had to be collected from different systems at different times because there was a difference in glochidia maturity in wild populations from those sites. Fishes from more northerly localities were exposed in mid-September (early-spring) and others from more southerly locations were exposed in mid-October (mid-spring) 2011. Mean temperatures were lower during the September exposure period than those in the October exposure period which may help explain why the former glochidia took 26-27 days to produce juvenile mussels and the latter fishes took 20-21 days to produce juveniles. Because I cannot separate the effects of fish species and post-hatching age of glochidia, I cannot say for certain that differences in transformation time were due to temperature alone, although preliminary findings are suggestive.

The findings of this study have a number of implications for the conservation of *W. carteri* and other Australasian hyriids which remain to be studied. First, some fish species may be more important than others in maintaining connectivity between patches of mussels. Although quantifying native fish migration patterns has been accomplished for

some species in the south-west of Western Australia (Chapman *et al*. 2006; Beatty *et al*. 2010b), more information will be useful in predicting the ability of host fishes to maintain connectivity between populations of *W. carteri*. The widely distributed surface feeding *Gal. occidentalis*, for example, is a strong swimmer (Pen & Potter 1991; Keleher 2010) and tends to travel great distances during annual spawning migrations; *Gal. occidentalis* could therefore be a key host species in maintaining connectivity among mussel populations. The relatively high prevalence of glochidia infestation on the euryhaline species, *A. suppositus, L. wallacei* and *Ps. olorum* could become detrimental to mussel populations in some cases. For example, if these fishes release metamorphosed juvenile mussels after they have migrated into more saline reaches of rivers, the survival of juveniles in these environments would be unlikely, given the low salinity tolerance of *W. carteri* (Chapter 2). In systems where estuaries connect to freshwater river reaches, the movement patterns of these species between fresh and saline waters are largely unknown and further information is needed to more accurately predict the fate of attached glochidia. Nevertheless, the gobies are common and found well inland from estuaries and are supported by breeding populations in freshwater rivers and lakes of the region (Morgan *et al*. 1998, 2011) and *Ps. olorum* is commonly reported as hosts for glochidia elsewhere (e.g. Walker 1981; Humphrey 1984; Widarto 1996).

5.5 Conclusions

Although host fishes have been identified for glochidia of a number of species in the Northern Hemisphere, the Southern Hemisphere fauna has received far less research attention (Walker *et al*. 2001). Furthermore, the majority of these studies have been on the identification of suitable hosts under controlled laboratory conditions (Strayer 2008), but

this study is one of the first to investigate glochidia infestation in wild populations of fishes (also see Kelly & Watters 2010). This study suggests fish species vary in their suitability as hosts based on the ability of *W. carteri* to metamorphose to the juvenile mussel stage. Like other Australian hyriids which have been studied, *W. carteri* appears to be a host generalist, capable of parasitising a variety of native/endemic hosts, four of which have been shown to produce metamorphosed juveniles in the laboratory, and includes at least one species of alien fish which supports glochidia metamorphosis. The only species (so far) which is almost certainly a non-host is the alien/introduced Goldfish. A number of other potential host fishes which occur in south-western Australia (see Morgan *et al*. 1998, 2011) remain to be tested for their ability to support the life cycle and thus the conservation of *W. carteri*.

Glochidia on infested fishes were approximately the same size and shape as those presented in Chapter 4. Broadly speaking, this study has shown that differences in prevalence and intensity occur that would be due to multiple factors in various river systems and host species. Further research is necessary to elucidate those factors that may influence the level of glochidia infestation, juvenile survival and preferred habitats and thus recruitment of *W. carteri* and other Unionoida.

Chapter 6

Intraspecific variability in growth and age in *Westralunio carteri*

6.1 Introduction

The dynamics of freshwater mussel populations are affected by variations in growth rates of individuals which can affect survival and reproduction (Hastie *et al.* 2000). The average growth rate and age-at-length estimates attained in threatened and harvested populations may be crucial in determining the overall reproductive output necessary for long-term population viability (Neves & Moyer 1988; Hastie *et al.* 2000). Generation length, determined from age at first reproduction, fecundity and survivorship is an important criterion for determining conservation status under the IUCN Red List Guidelines (IUCN 2011).

Freshwater mussels generally have prominent concentric rings in their shells, analogous to growth rings in trees, otoliths in fishes or teeth in vertebrates (Clark 1974; Haag 2009). Growth lines, synonymous with growth 'rings', 'bands', 'checks' (Neves & Moyer 1988) or 'rests' (McQuaig & Green 1983) are formed during interruptions in growth arising from any number of environmental changes (Clark 1974). For example, extreme changes in temperature limiting metabolic activity is a common cause for growth interruption and produces regular periodic darkened growth interruption lines in temperate seasonal climates particularly in northern latitudes of Europe and North America (e.g. Neves & Moyer 1988; Schöne *et al.* 2004; Howard & Cuffey 2006; Haag & Commens-Carson 2008). The formation of shell rings on an annual basis (i.e. 'annuli') has been validated in many species over multiple years in various habitats in Europe and Russia (Hastie *et al*., 2000; Ziuganov *et al*., 2000; San Miguel *et al*., 2004; Schöne *et al*., 2004; Helama

et al., 2006; Helama & Valovirta, 2008), Israel (Ostrovsky *et al.* 1993), North America (Hanson *et al*., 1988; Neves & Moyer, 1988; Howard & Cuffey, 2006; Haag & Commens-Carson, 2008; Haag, 2009; Haag & Rypel, 2011), South America (Parada *et al.* 1989) and Japan (Kondo 1992; Negishi & Kayaba 2009, 2010).

Early work on age estimation was derived from counting external growth rings (Lefevre & Curtis 1912; Isley 1914; Coker *et al.* 1921; Chamberlain 1931; Stansbery 1961). Problems have arisen from attempts to determine age from external growth rings due to eroded shell surfaces, obscured rings on darkly coloured shells, subjectivity in distinguishing annuli from stress-induced rings and the difficulty in counting closely spaced rings near the ventral margin of older mussels (Ansell 1968; Coon *et al*. 1977; Lutz & Rhoads 1980; Neves & Moyer 1988). The technique of counting internal growth rings from thin sections of shells has long been used in marine bivalves and provides a more robust estimation of growth ring counts than external ring counting (Neves & Moyer 1988).

Alternative methods of age estimation have also been useful in marine bivalves, including the use of stable oxygen isotopes (Krantz *et al*. 1984) and uranium-series radioisotope dating (Turekian *et al*. 1975, 1979). These methods are, however, contingent on significant annual growth increments which are often lacking in freshwater mussels (Anthony *et al.* 2001). Average individual growth rates have been estimated by fitting asymptotic von Bertalanffy curves (von Bertalanffy 1938) to age-at-length estimates (e.g. Alimov 1981; Ross 1984, 1988; Bauer 1991, 1992; Semenova *et al.* 1992; Ziuganov *et al*. 1994; Beasley 1996; Hastie *et al*. 2000; Anthony *et al*. 2001), a method which has been widely used in

fisheries research (Hastie *et al*. 2000). In Anthony *et al.* (2001), the authors proposed an algebraic reorganization of the von Bertalanffy growth equation to solve for age when other parameters of the equation are estimated from linear regression analyses, such as the Ford-Walford relationship (Ford 1933; Walford 1946) which utilises mark-recapture growth data (e.g. Ricker 1975). Haag (2009) subsequently corrected errors in the equation presented by Anthony *et al.* (2001) and evaluated how the range and skew of observed mark-recapture data can affect growth parameter estimates, how growth reduction from handling can affect age estimates and compared estimates of growth derived from validated shell rings with growth observed from mark-recapture data.

Very few age and growth studies have been published from the Southern Hemisphere (Walker *et al.* 2001) and there is virtually nothing known about growth and age estimation in the Australasian hyriids, with the exception of some data from Walker (1981) and Humphrey (1984). This is the first thorough examination of growth and age estimation in *W. carteri*. The aim of the current study is to determine the growth of *W. carteri*. Given that south-western Australia has a predictable temperate climate and that annuli have been validated in otoliths of fishes (e.g. Pen & Potter 1990, 1991; Morgan *et al*. 1995, 2000, 2002) which co-occur with *W. carteri* (see Chapter 5), I hypothesise that internal growth interruption lines occur annually in the shells of *W. carteri.*

6.2 Materials and methods

6.2.1 Study area

In order to determine and compare the growth rates of *W. carteri* within a number of perennial rivers in south-western Australia, mark-recapture of individuals (N = 366) occurred between 10 February 2010 and 25 February 2011 in five populations occurring in spatially and qualitatively distinct habitats, within the region (Figs. 6.1 – 6.2).

Environmental summary data for each locality was obtained from chemical water parameters downloaded from the Western Australian Department of Water gauging station Water Resources Database (http://kumina.water.wa.gov.au/waterinformation/wrdata/wrdata.cfm), which are presented in Table 6.1. The populations were:

1) **Bennett Brook**: (31°52`43``S, 115°57`35``E) A tributary of the Swan River; the brook is maintained by a mound spring; located in a rehabilitated conservation reserve surrounded by urban developments; sediments were mostly coarse sand with fine black silt along the banks; riparian vegetation was weedy in the understorey with relatively abundant *Eucalyptus* and *Melaleuca* spp. which provided ample shade.
2) **Brunswick River** (33°13`33``S, 115°55`40``E): On private rural residential property; heavily shaded by *Eucalyptus* trees with dense foliage but the understorey mostly grassy and weedy; in stream habitat dominated by small woody debris, leaf matter and muddy sand substrates.
3) **Collie River** (33°18`5``S, 115°48`58``E): Land use is primarily agricultural with an irrigated turf farm and rural residential property on either side of the

river; the river is maintained by flow releases from the Wellington Reservoir 18-20 km upstream. Riparian zone dominated by grassy understorey with intermittent *Eucalyptus* trees and occasional shrubs; stream banks primarily grassy with some patches of reeds; substrates composed primarily of sand with silt in areas of low flow and mud along the banks; large woody debris relatively common and ranging in size from large logs to thick tree branches; leaves and detritus accumulate in low flow areas.

Serpentine River: Two very different sites were utilised for the growth trials.

4) The **Dog Hill** site (32°20`32``S, 115°51`39``E): Located ~2 km downstream from the confluence of the Serpentine River and Birrega Drain. Riparian vegetation is mostly grassy weeds with very few trees or shading vegetation; in-stream habitat is primarily bedrock with patches of sand and silt which collected in shallow depressions along the banks; dense algal mats are present during the summer.

5) The **Horse Drink** site (32°20`00``S, 115°54`14``E): Located within a privately held conservation reserve with an overstorey composed of relatively large eucalypt trees which provide ample shade; a patchy middlestorey with shrubs and woody climbers and a patchy understorey with a mixture of small native plants and introduced weeds; in-stream habitats are complex with a variety of macrophytes, large and small woody debris, overhanging vegetation along the banks, exposed tree roots, many bends and side channels, riffles and pools; substrates were mostly sandy with a mixture of mud and silt along with leaf matter.

Table 6.1 Summary data (1974-2009) for environmental parameters of water quality for localities in which *Westralunio carteri* was sampled for mark-recapture growth studies and quantified studies of internal shell rings to estimate ages-at-length. Abbreviations: DO = dissolved oxygen; TN = total nitrogen; TP = total phosphorus

		Salinity (g L^{-1})		Temperature (° C)		pH		TP (mg L^{-1})		TN (mg L^{-1})		DO (%)	
Site	Corresponding AWRC gauging station	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Bennett Brook	616084 (31°52`39``S, 115°57`34``E)	0.44	0.10 - 1.20	18.4	11.4 - 24.4	7.1	6.9 - 7.6	0.12	0.01 - 1.20	0.47	0.47 - 6.30	61.1	41.6 - 77.0
Brunswick River	612022 (33°13`10``S, 115°55`18``E)	0.21	0.00 - 0.70	14.6	8.5 - 25.5	7.3	5.0 - 9.8	0.49	0.40 - 2.10	0.81	0.5 - 3.5	82.4	32.9 - 134.2
Collie River	612043 (33°17`57``S, 115°47`58``E)	1.10	0.39 - 3.16	17.5	9.1 - 28.5	7.2	5.5 - 8.9	0.15	0.01 - 1.83	0.56	0.14 - 2.93	81.7	0.0 - 259.4
Dog Hill	614030 (32°20`30``S, 115°51`41``E)	0.43	0.10 - 2.89	19.1	9.0 - 35.2	7.0	5.7 - 9.1	0.27	0.01 - 2.4	1.73	0.15 - 16.0	96.0	30.0 - 174.5
Horse Drink*	614114 (32°20`13``S, 115°53`06``E)	0.29	0.19 - 0.41	16.7	11.7 - 24.8	7.1	6.6 - 7.7	0.06	0.03 - 0.13	0.85	0.23 - 2.0	67.9	44.4 - 85.6

*DO data from Klunzinger *et al*. 2011c

6.2.2 Annuli validation

I used calcein as an *in situ* growth marker to validate the occurrence of annuli and compared estimates of age by counting annuli with estimates derived from mark-recapture growth data.

To test whether the formation of internal shell rings occur annually and thus whether they represented true annuli, two samples (n = 17 and 34) of *W. carteri* of various sizes from the Collie River were labeled with individually numbered plastic tags, glued to the external surfaces of the shells and then immersed in a solution of 250 mg L^{-1} calcein (SE-MARK®, Western Chemical, Ferndale, WA 98248, U.S.A.) for 24 h at 5° C on 24 December 2009 and 11 March 2010, respectively. This concentration has previously been shown to be effective in marking freshwater mussel shells (Eads & Layzer 2002). The mussels which had been treated with calcein were recaptured on 17 February 2011 and dissected and sectioned. The presence of a single fluorescent line (observed using a compound microscope with an Interference Blue Filter for 495/520 Excitation/Emission wavelengths) between two growth disturbance rings in thin shell sections would validate whether the rings formed on an annual basis.

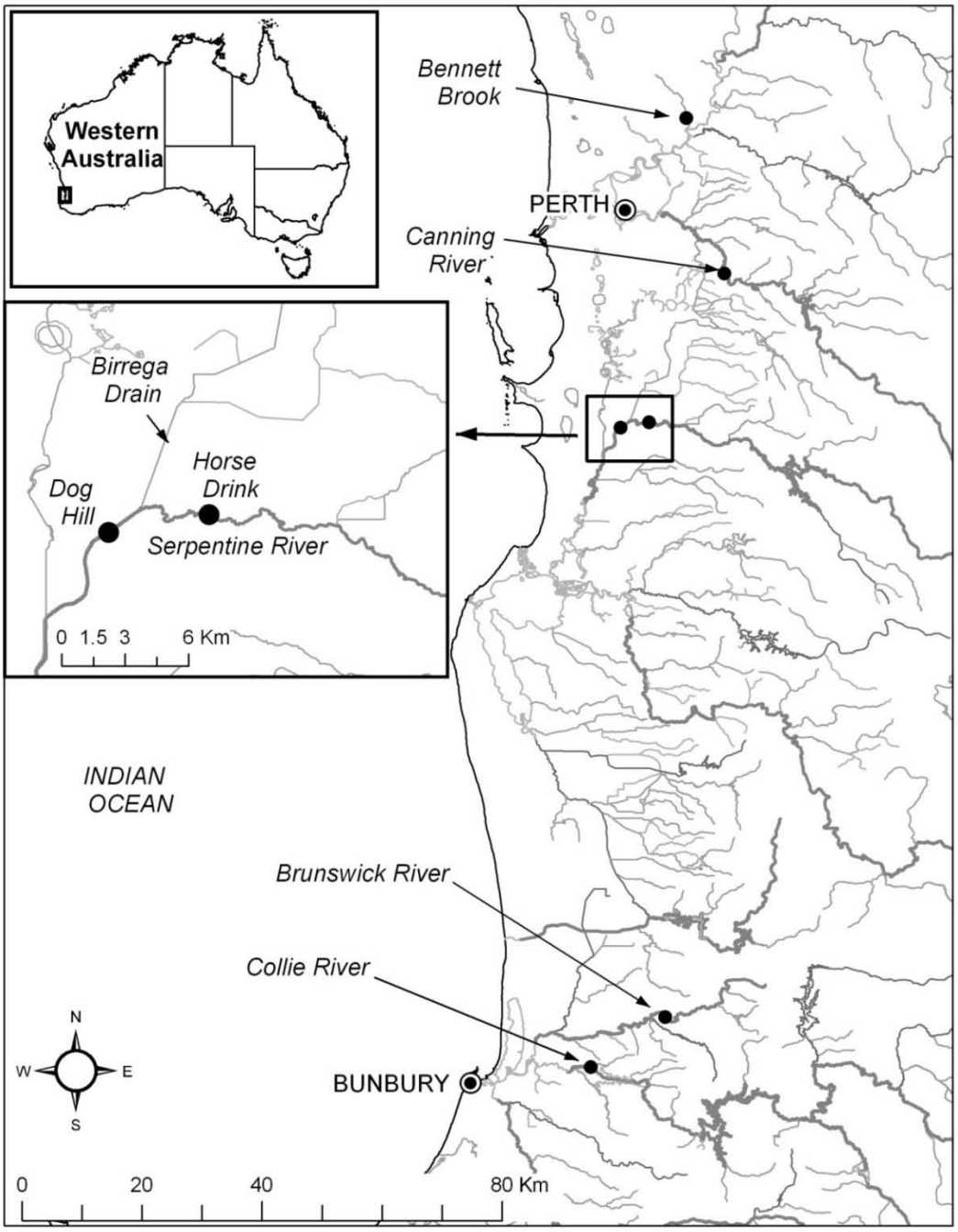

Fig. 6.1 Location of populations of *Westralunio carteri* sampled for mark-recapture growth experiments and age estimates using annuli counts. (Spatial data provided by Western Australian Department of Water, under license).

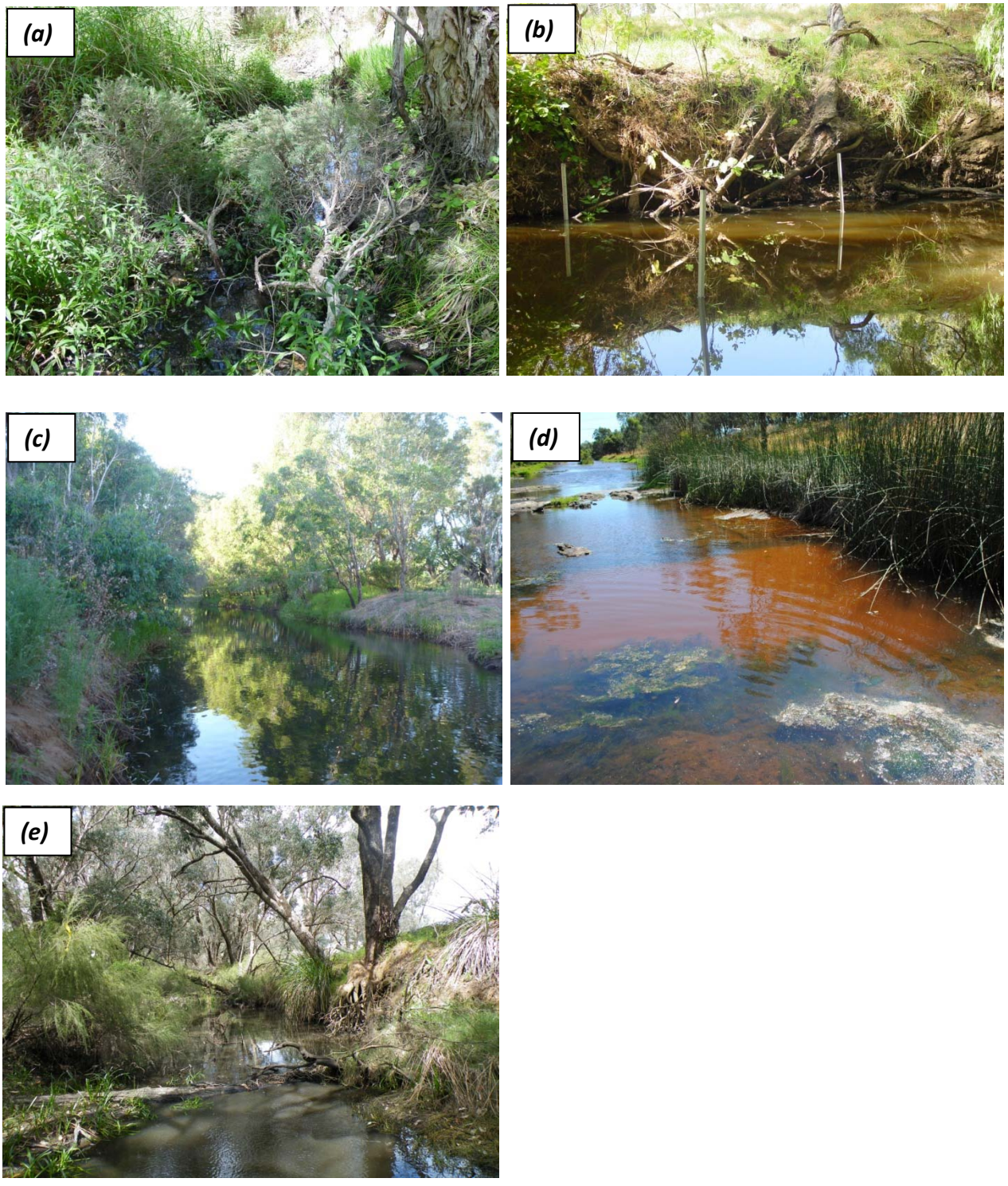

Fig. 6.2 Examples of habitats sampled for growth and age studies of *Westralunio carteri*. Photos were taken in February 2010. *(a)* Bennett Brook; *(b)* Brunswick River; *(c)* Collie River; *(d)* Serpentine River (Dog Hill); *(e)* Serpentine River (Horse Drink).

6.2.3 Age estimation from shell annuli

Mussels were sampled from each of the five populations, transported live in plastic buckets containing river water and subsequently anaesthetised in ice slurry and dissected in the laboratory. Soft tissues were removed and gender for each individual was determined by the presence (females) or absence (males) of marsupia on the inner two thirds of the inner demibranchs of the gills. Shells were prepared for thin sectioning using methods similar to those described by Haag & Commens-Carson (2008). Shells were lightly wiped clean with river water and dried. To avoid shell splintering when being sliced, shells were first coated with FR 251 epoxy resin (Fiberglass Resin & Sales, Welshpool, Western Australia 6106) from one side of the umbone to the ventral edge and subsequently cut using a rotary cutting tool equipped with an abrasive cut-off wheel (23.8 mm diameter, 1.0 mm thick). The cut edge of the shell was then moistened with water and polished on a flat surface with 600 grit wet/dry sandpaper in a figure eight pattern until the cut surface was flat and within 0.5 mm of the umbone. The cut and polished shell section of each specimen was then coated with resin and mounted firmly on a glass microscope slide coated with resin, cut side down and allowed to harden for 24 hours and subsequently sliced to a thickness of ~0.8 mm using a low speed sectioning saw (Isomet® 111280, Buehler, Lake Bluff, IL, USA 60044) equipped with a diamond wafering blade (127 mm diameter, Isomet® 114245, Buehler, Lake Bluff, IL, USA 60044) and a glass slide chuck (BUE11-2488, Buehler, Lake Bluff, IL, USA 60044) at 210 revolutions per minute. From shell annuli data, growth interruption line counts were plotted against shell length and curves were fitted to the scatter plots for each population using best fit analysis in Sigma Plot 12.0.

Thin sections of shells were observed in random order. To avoid bias, the only information available to the investigator for each individual shell section at the time of reading was the plastic tag number. Shell sections were observed under a light microscope and the number of growth interruption lines counted from the base of the umbone outward to the ventral edge of the shell for each specimen.

6.2.4 Growth modelling

On each initial marking occasion, sub-samples of *W. carteri* were hand-collected from study sites; shells were measured for length (*L*) following McMichael & Hiscock (1958) to the nearest 0.01 mm. To reduce the stress of handling, mussels were maintained in plastic buckets containing river water in cool, shaded areas while they were measured. Care was taken not to damage the shells of the captured mussels.

For identification of mussels, an individually numbered 8 x 4 mm shellfish tag (Hallprint Pty Ltd, Hindmarsh Valley, SA 5211, Australia) was glued to the left valve surface of each mussel with Super Glue® (Super Glue Corp., Rancho Cucamonga, CA 91730, U.S.A.). Since growth rates of freshwater mussels have been demonstrated to be altered by excessive handling (e.g. Haag 2009), marked and tagged mussels were left *in situ* for a period of approximately one year before being recaptured and measured for *L* to the nearest 0.01 mm.

Growth rates of each population were quantified and parameters of the von Bertalanffy growth equation (von Bertalanffy 1938) were estimated from the Ford-Walford (Ford 1933; Walford 1946) relationship. Estimates of L_∞ and K were undertaken using the equations:

$$L_{t+1} = a + bL_t \quad (1)$$

where L_{t+1} is the length after time t plus 1 year and L_t is the initial length at time t. The parameter a is the y-axis intercept and b is the slope of the regression and:

$$L_\infty = \left(\frac{a}{(1-b)}\right) \text{ and } \quad K = -\ln b \quad (2)$$

To determine whether the mean growth rate of *W. carteri* differed significantly between populations, the difference in mean growth increments of mark-recaptured individuals was tested by employing analysis of covariance (ANCOVA) in a General Linear Model with L as the co-variate. A Levine's test for equality of error variance was initially undertaken on the un-transformed data. Heteroscedastic data were transformed prior to ANCOVA being undertaken. Spearman's Rank Order analysis tested whether growth rate was correlated with L. All statistical tests were undertaken using PASW Statistics v18.

To estimate age from the Ford-Walford estimates of the von Bertalanffy parameters, I applied the inversion of the von Bertalanffy equation reported by Haag (2009) as the following:

$$t = \frac{\ln\left(\frac{(L_\infty - L_t)}{(L_\infty - L_0)}\right)}{-K} \quad (3)$$

where L_t is the mussel length at time t (age in years), and L_0 is length at time = 0. From Chapter 4, I assumed a known mean glochidia length for *W. carteri* as 0.308 mm for L_0.

6.3 Results

6.3.1 Validated annuli

Calcein was effective in validating annuli in all of the mussels recovered (n = 19) from the Collie River. Growth interruption lines appeared as fluorescent green lines when shell sections were examined under blue excitation microscopy (Fig. 6.3). Annuli counts in shell sections from five populations of *W. carteri* are presented in Fig. 6.4. Two-parameter simple exponent models were the best fit for the data. Using this method of age estimation, assuming growth rings follow my validation procedure, ages at maximum shell lengths ranged from 36 years in the Serpentine River (Dog Hill) to 51 years in the Brunswick River at shell lengths of 82.8 and 71.8 mm, respectively. Age-at-length estimates from annuli counts were significantly different between populations (F = 9.48; d.f. = 4, 429; P < 0.001). Actual growth rates and age-at-length estimates were not different (ANCOVA, P > 0.14) between male and female *W. carteri* for any of the populations.

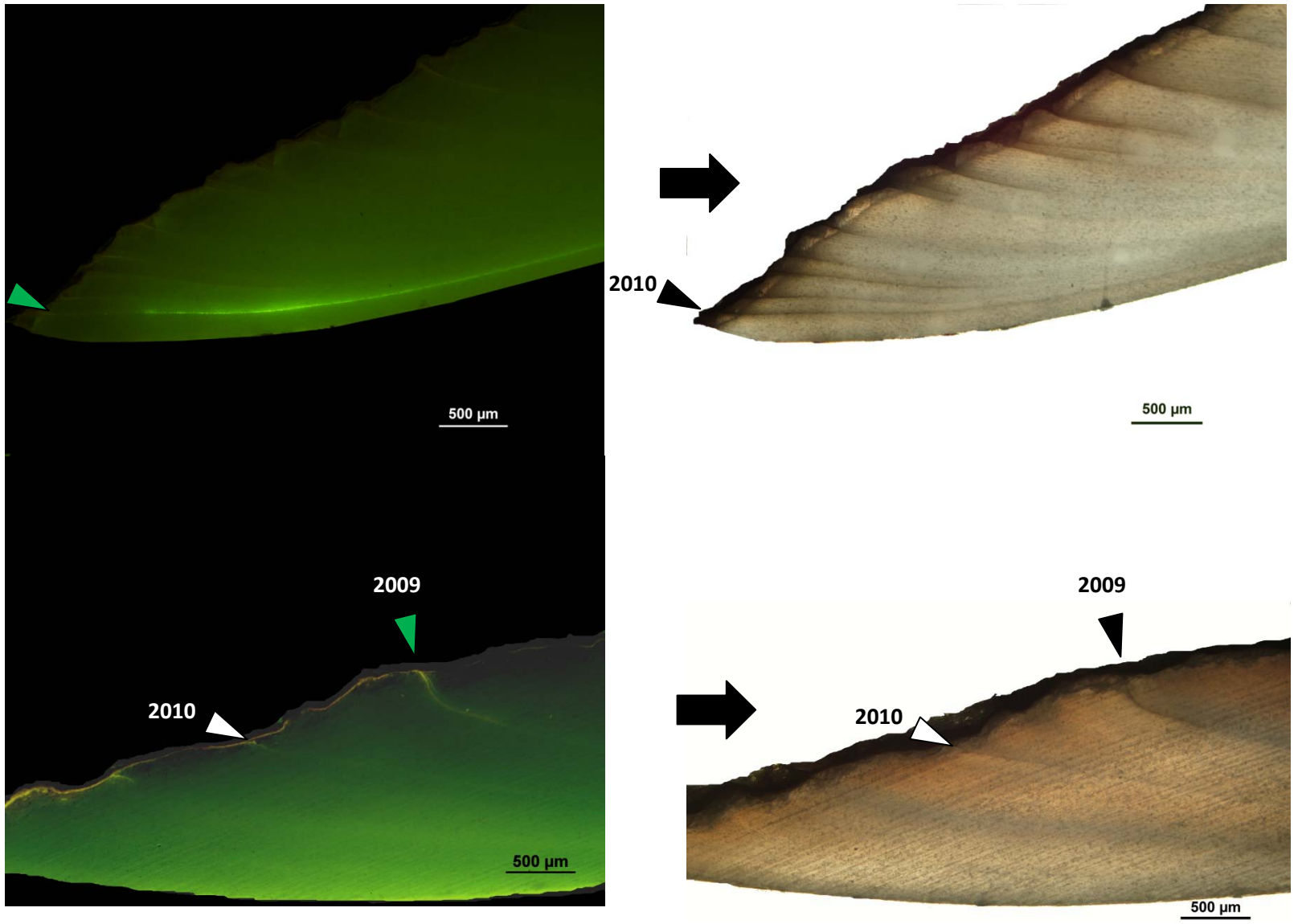

Figure 6.3 Thin section of the medial portion of the left valve of *Westralunio carteri*. Top images show validated annuli, one year after calcein exposure (arrows) under blue excitation (left) and white (right) light microscopy. Bottom images show validated annuli, two years after calcein exposure (black and green arrows) under blue excitation (left) and white (right) light microscopy. The annulus formed one year after calcein uptake in the bottom images is indicated by the white arrows.

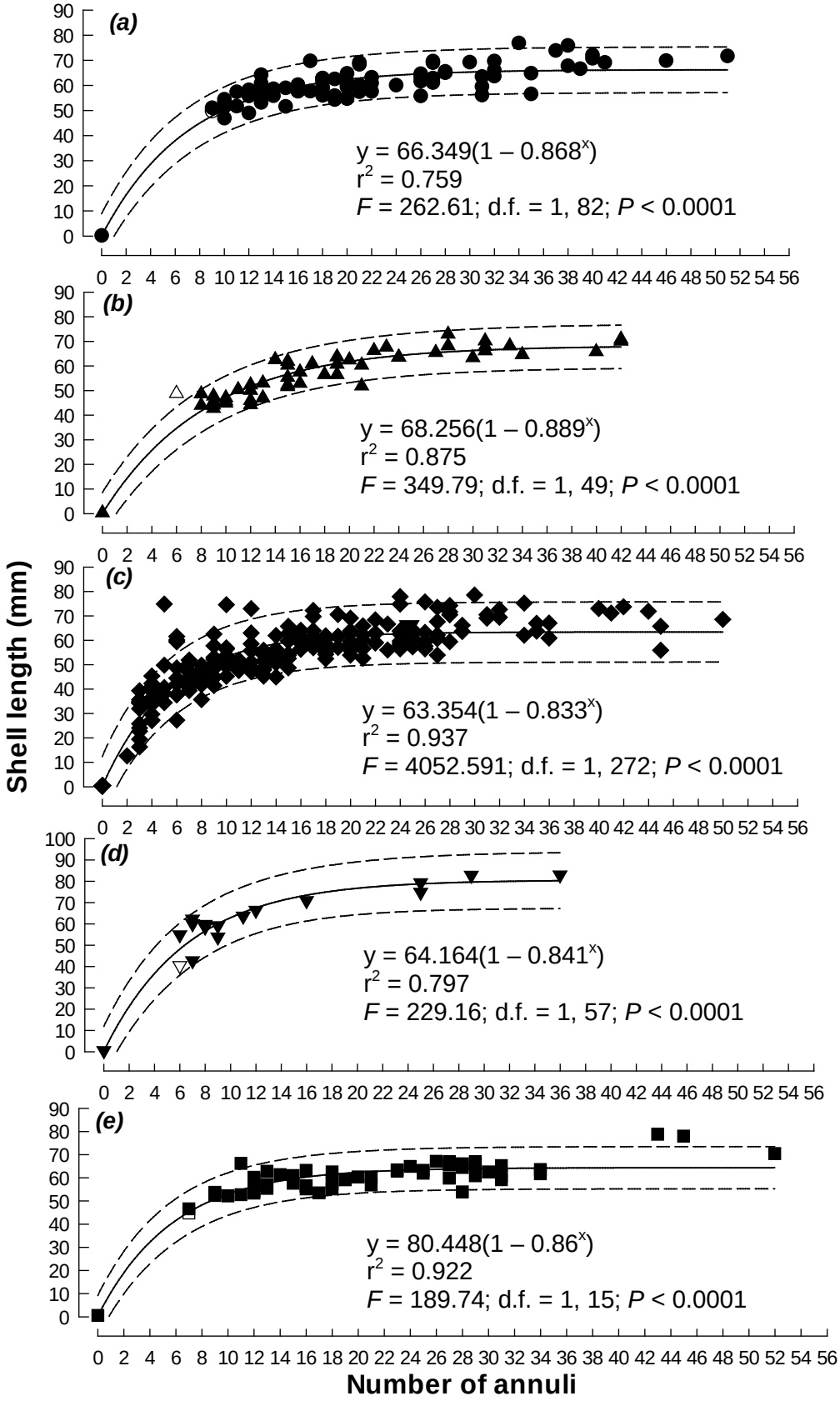

Fig. 6.4 Age estimates for *Westralunio carteri* within *(a)* Bennett Brook, *(b)* Brunswick River, *(c)* Collie River, *(d)* Dog Hill and *(e)* Horse Drink. Ages estimated from counting the number of annuli in thin shell sections. 95% Confidence prediction bands are shown as dashed lines.

6.3.2 Growth rates and estimation of von Bertalanffy growth parameters

The recapture rate of tagged *W. carteri* was 71.4, 90.7, 44.8, 48.6 and 70.8% in Bennett Brook, Brunswick River, Collie River, Dog Hill and Horse Drink populations, respectively. Mean size of mussels in the mark-recapture program ranged from 55.47 (±0.71) mm *L* in the Collie River to 64.25 (±3.18) mm *L* in the Serpentine River at the Dog Hill site. Mean growth rates of mark-recaptured individuals ranged from 0.36 (±0.05) mm yr^{-1} in the Brunswick River to 3.82 (±1.16) mm yr^{-1} in the Serpentine River at the Dog Hill site (Table 6.2). Differences in mean growth increments between sites were highly significant (ANCOVA, $P < 0.001$).

The Ford-Walford estimation of von Bertalanffy parameters also revealed considerable variation in growth rates of *W. carteri* with the growth constant *K* ranging from 0.05 in the Brunswick River to 0.26 in the Serpentine River (Dog Hill). Theoretical L_∞ ranged from 61.18 mm in the Collie River population to 76.57 mm in the Serpentine River (Dog Hill) (see Fig. 6.5, Table 6.2). Growth was negatively correlated with *L* within each population and overall (Table 6.3).

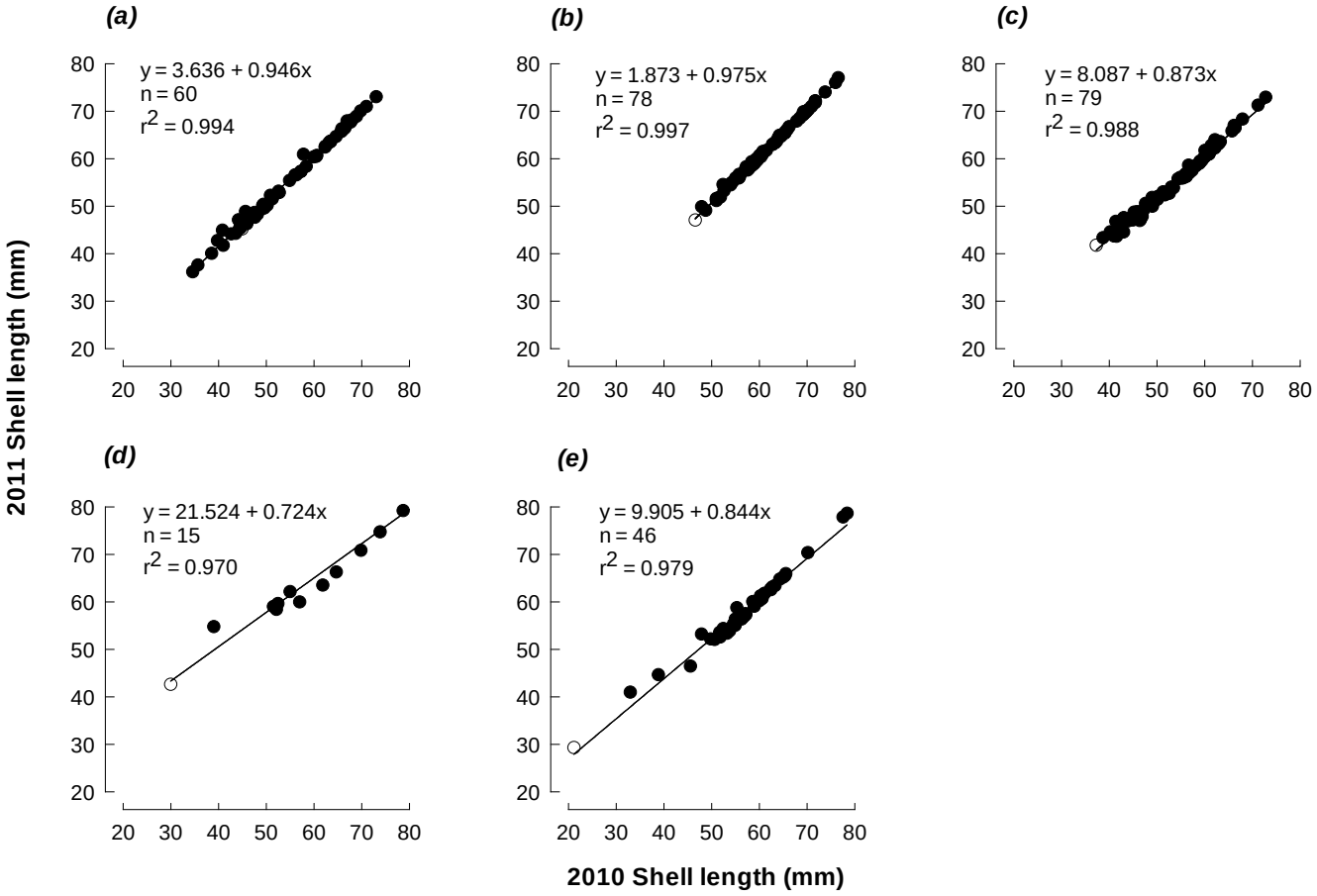

Fig. 6.5 Ford-Walford growth plots of *W. carteri* from the five populations sampled during the 2010-2011 mark-recapture period. (a) Bennett Brook; (b) Brunswick River; (c) Collie River; (d) Serpentine River (Dog Hill); (e) Serpentine River (Horse Drink).

Table 6.2 Growth parameters for *W. carteri* based on Ford-Walford estimates of mark-recaptured individuals from sites sampled in south-western Australia

Site	**Number of marked individuals**	**Mean *L* (±s.e.)**	***a***	***b***	**Mean (±s.e.) growth rate (mm yr^{-1})**	***K*** **(yr^{-1})**	L_∞ **(mm)**
Bennett Brook	60	55.34 (1.23)	3.835	0.942	0.69 (0.12)	0.06	66.12
Brunswick River	78	61.51 (0.74)	3.014	0.954	0.36 (0.04)	0.05	65.52
Collie River	79	55.47 (0.71)	8.626	0.859	1.02 (0.13)	0.15	61.18
Serpentine River (Dog Hill)	15	64.25 (3.18)	17.458	0.772	3.82 (1.16)	0.26	76.57
Serpentine River (Horse Drink)	46	57.70 (1.21)	9.972	0.842	1.08 (0.28)	0.17	63.11

Table 6.3 Correlation analysis of length and growth rates in five populations of *Westralunio carteri* during the 2010-2011 mark-recapture period.

Site	Spearman's correlation coefficient	*P*-value
Bennett Brook	-0.812	< 0.001
Brunswick River	-0.466	< 0.001
Collie River	-0.791	< 0.001
Serpentine River (Dog Hill)	-0.982	< 0.001
Serpentine River (Horse Drink)	-0.541	< 0.001
Overall	**-0.654**	**< 0.001**

6.3.3 Age estimates from growth data

From the Ford-Walford estimates of L_∞ and K and the inversion of the von Bertalanffy growth equation, age at length estimates are presented in Fig. 6.6. Predicted ages for theoretical maximum shell sizes ranged from 25 years old in the Serpentine River (Dog Hill) to 105 years old in Bennett Brook. The ages at maximum length estimated from growth data differed markedly from those estimated by counting shell annuli in each population (Table 6.4).

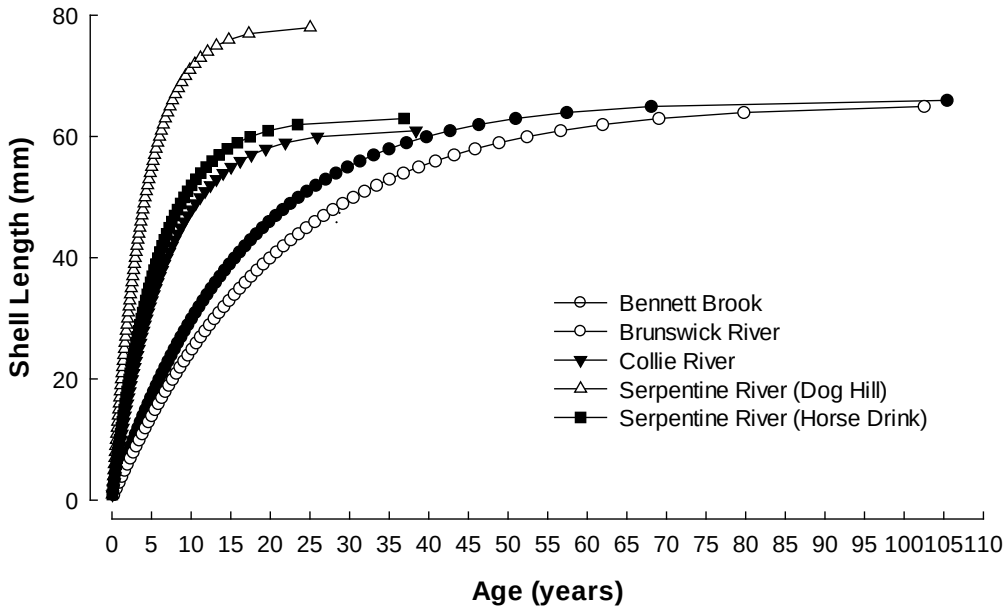

Fig. 6.6 Theoretical age estimates for *Westralunio carteri* estimated from the inversion of the von Bertalanffy growth equation using growth parameters derived from the Ford-Walford relationship.

Table 6.4 Comparison of maximum ages-at-length for *Westralunio carteri* from five populations in south-western Australia, between those predicted from growth data and those determined from annuli counts.

Site	Growth data		Annuli counts	
	Maximum length	Maximum age	Maximum length	Maximum age
Bennet Brook	66	105	70.9	42
Brunswick River	65	102	71.8	51
Collie River	61	38	68.5	50
Serpentine River (Dog Hill)	78	25	82.8	36
Serpentine River (Horse Drink)	63	37	78.6	43

6.4 Discussion

6.4.1 Validation of annuli

This is apparently the first study to utilise calcein to validate annuli in adult freshwater mussels. Calcein marking proved to be an effective method to demonstrate that growth interruption lines within the shells of *W. carteri* occur annually. Although annuli have been validated for more than 20 species of freshwater mussels (Neves & Moyer 1988; Howard & Cuffey 2006; Haag & Commens-Carson 2008), pseudo-annuli (resulting from handling) have appeared as non-annual growth rings from other studies (Downing & Downing 1992; Downing *et al*. 1992; Kesler & Downing 1997). I also note that the latter studies were performed on the northern edge of distributional range boundaries in seasonably cold Canadian Lakes with limited calcium budgets, which could have also affected the formation of pseudo-annuli (cf. A.E. Bogan in the examination of this thesis, December 2012). The aim of using calcein as a growth marker for validation was to minimise the effect of handling on producing extra non-annual rings, which was demonstrated as a non-invasive method for measuring growth in juvenile freshwater

mussels of North America (Eads & Layzer 2002) and more recently in young bivalves of Mauritania (van der Geest *et al*. 2011).

Given that the shells of *W. carteri* are relatively uniform in colour and often appear to be 'smooth' from mud deposits, thus rendering external growth interruption lines difficult to see, and because calcein validated internal growth interruption lines as annuli in all of the individuals which were recaptured, their use as an aging technique is justified. Thus, validated annuli counts probably offer the most realistic age-at-length estimates for growing populations of *W. carteri*. Future studies should validate estimates for other populations of *W. carteri*, as has been suggested or demonstrated for other species elsewhere (Walker 1981; Neves & Moyer 1988; Schöne *et al*. 2004; Haag & Commens-Carson 2008; Haag 2009).

Growth interruption lines or pseudo-annuli have been known to occur less than annually from environmental stressors, so interpretation of growth lines should be framed cautiously (Neves & Moyer 1988; Walker *et al*. 2001). However, Haag & Commens-Carson (2008) demonstrated the introduction of pseudo-annuli from stress in which individuals were handled several times over the course of a year. To avoid this bias, I only handled *W. carteri* twice: once upon initial measurement and once upon recapture.

Growth interruption lines may also arise however from events such as aestivation and hypoxic stress, physical injury by predators, storms, floods, droughts, abnormally high temperatures and anoxia (Clark 1974; Lutz & Rhoads 1980; Walker *et al*. 2001; Haag & Commens-Carson 2008). Some authors have been able to discern these 'false' rings or 'pseudo-annuli' easily (Chamberlain

1931; Negus 1966; Day 1984), while others have had limited success, particularly in riverine species (Coon *et al.* 1977; Haukoja & Hakala 1978).

6.4.2 Comparison of annuli and growth models to estimate age

Although actual growth rates were negatively correlated with age and L within all populations and overall, estimates of K and L_∞ from the Ford-Walford plots and von Bertalanffy growth equation indicated no relationship. Furthermore, estimates of L_∞ (maximum shell length) and maximum age were different from what I observed in actual maximum L and maximum age inferred from annuli counts, which agrees with Haag (2009).

Estimates of age from mark-recapture growth models, as shown here and by other authors (e.g. Anthony *et al*. 2001; Haag 2009) may introduce a number of biases and can be substantially different from ages-at-length estimated from annuli counting. The effects of handling, proportion of various size classes being tested, the assumption of asymptotic growth, and the characteristics of the growing season in which the individuals are tested have been shown to introduce inaccuracies between ages predicted from mark-recapture data and those observed by counting validated annuli (Haag 2009; Haag & Rypel 2011).

6.4.3 Growth rates and longevity

Like other studies (see review by Haag & Rypel 2011), the growth curves from my mark-recapture data show that growth is more rapid in the smaller (younger) size classes and slows to near zero growth in the largest size classes of *W. carteri*. Thus, the data are useful in showing that *W. carteri* potentially lives for at least 36 to 52

years; growth is rapid in the first four to six years and slows with size. What remains unanswered is the problem of estimating actual longevity. If growth is asymptotic, which most of the literature suggests, if shell shrinkage occurs after maximum *L* is attained in older individuals and if annuli are discontinuous or indistinguishable in these older individuals, how can we estimate longevity for certain? Short of tracking growth and age over a mussel's lifetime, longevity may be a next to impossible task to undertake, particularly if they live as long as the extreme cases in colder climates such as northern Europe (*M. margaritifera*, for example, is estimated to live for more than 200 years; Schöne *et al*. 2004).

Downing & Downing (1993) were first to draw attention to the issue of shell shrinkage in large freshwater mussels. Most authors have dismissed negative growth as apparent 'measurement error' (Downing & Downing 1993). The fact that the phenomenon has occurred over widely disjunct populations (Downing *et al*. 1993) separated by geography and by several different authors suggests that perhaps older mussels may indeed incur negative growth. Although not presented here, I did occasionally find that large *W. carteri* had apparent negative growth in each of the populations, which could indicate actual shrinkage of shells (Downing *et al*. 1992; Downing & Downing 1993; Haag 2009), but because this would have led to gross underestimates of maximum sizes from the Ford-Walford relationship, I assumed zero growth in these individuals, as have other authors (Haag 2009; Haag & Rypel 2011).

McMahon & Bogan (2001) suggest that although growth may still be apparent as some individuals near maximum length, shell matter continues to accumulate on the inner surface of the shell, resulting in increased mass with age.

Although this may be true for some species, *W. carteri* and other Australian hyriids (e.g. Walker 1981) often have extremely eroded shells, which would render the use of this method unsuitable.

6.4.4 Differences among populations

Growth rates, age estimates and maximum shell lengths varied significantly among populations, which was probably due to a number of factors such as genotypic variation, differences in physical habitat (such as substrate type, hydrology, riparian vegetation and hydrography), hydrochemistry and temperature (Bjork 1962; Alimov 1981; Dyk & Dykova 1974; Eagar 1977; Tevesz & Carter 1980; Walker 1981; Bauer 1991, 1992; Hruška 1992; Semenova *et al*. 1992; Beasley 1996; Morris & Corkum 1999; Hastie *et al*. 2000; Valdovinos & Pedreros 2007; Haag & Rypell 2011). The mussels from the heavily eutrophied, warmer Birrega Drain within the Serpentine River catchment grew faster and were much younger than similar-sized *W. carteri* from other populations, which was likely due to a history of high nutrient export within the catchment. Indeed excessive growth of benthic alga and fish kills within the Peel-Harvey Estuary was attributed to nutrient pollution, 90% of which was accredited to the heavy use of superphosphate fertilizer within coastal plain catchments including the Serpentine River (Birch 1982), a legacy which continues today (Tweedley *et al*. 2012). I was not, however, able to attribute water chemistry, temperature or habitat directly to the observed differences in annuli counts due to incomplete datasets among sampling sites.

6.5 Conclusions

Determination of generation length is one of the criteria used for assessing the conservation status of a taxon (IUCN 2011). The present study is a start towards that determination for *W. carteri*. From Chapter 4, I identified the size of glochidia, which I used for the length at age 0. From Chapter 3, the smallest *W. carteri* which were reproductively active were 26.7 mm from the Collie River. Applying the age-at-length relationship calculated in this chapter suggests that the age at first reproduction is two to three years old. The data contained in this chapter also suggest that *W. carteri* live for at least 50 years.

From a conservation management perspective, knowing that individuals take two to three years to mature and that the loss of large, old individuals within a population may take many years to recover, may have serious consequences for the viability of the population. Furthermore, the longevity of the species indicates that populations may appear to be stable even if recruitment has not occurred for a long period of time; a scenario referred to as 'extinction debt' (Strayer 2008). More work is needed to determine the level of recruitment, fecundity, age, population composition and survivorship throughout the distributional range to determine the conservation needs and predict the consequences of continuing decline for this threatened species.

Chapter 7

Summary of results and implications for future research

7.1 Conservation status of *Westralunio carteri*

Clearly, like other freshwater fauna of south-western Australia, *W. carteri* has undergone major declines in terms of distributional range arising from salinity problems and water loss. The original nomination of the species as Vulnerable and the decision to change the species' status to Least Concern is questionable given the lack of published quantitative data on the species' distribution and tolerance to threats. The species should have been listed as 'Data Deficient', as has been the case for other nominated Australian hyriids, given that most, if any IUCN Red List Guidelines could not be assessed accurately.

The results contained in this study, however, demonstrate that nominators were probably on the right track when originally suggesting Vulnerable, despite the lack of published information and that, given the new information presented here, I suggest an 'Endangered' listing citing salinity and lack or loss of perennial water resources as the primary causes of decline in Extent of Occurrence and are the major threatening processes to the species' survival. Assessors will also, of course, need to recognise that other threats such as interstitial ammonia from nutrient pollution, low pH from acid sulphate soils and possibly excessive acid runoff from plantation forestry, crushing and erosion from livestock, sedimentation from development, barriers to host fish movement, invasive species, parasitism and disease, feral pig predation and others are probably causing localised declines in abundance which may result in further range declines if not managed effectively.

Indeed, I witnessed the effects of salinisation on several populations of *W.*

carteri, which resulted in complete localised extirpations in the main channel of the Blackwood River, an 8 km stretch of the Lower Canning River and Yule Brook, the Murray River, and the mid-channel of the Warren River as well as major losses associated with dewatering, drought and summer-time stagnation of riverine pools. The sound distribution data along with the Extent of Occurrence, determined from IUCN guidelines, paints a clear picture of the species' decline in the last 50-100 years. This thesis should be followed with a justified nomination for the species to be listed as Endangered, or at the very least Vulnerable under the Western Australian *Wildlife Conservation Act 1950* and the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* and internationally on the *IUCN Red List of Threatened Species*.

7.2 Life history of *Westralunio carteri*

From this study, we now know several aspects of the life cycle of *W. carteri* with some certainty. We know that, like freshwater crayfish (Beatty *et al*. 2003) and fishes of the region (Morgan *et al.* 1998, 2011), reproduction is highly seasonal, with spawning occurring during the winter rainy season in June and July, followed by a brief brooding period as winter flows subside and the release of glochidia on mucus strings as water temperatures become warmer.

Glochidia are comparatively larger than several of the other Australasian hyriids that have been studied and distinct enough in shape and larval tooth morphology to be distinguished from other species. This study stems from a rather fortuitous discovery of cysts on Freshwater Cobbler, during another study in the Blackwood River by previous postgraduates and my supervisors. It is odd that

these white pustules, containing glochidia, seem only to occur on this species of fish and not the other species of fish I found infested with glochidia. Indeed glochidia are unrecognisable on other species of host fishes without a really close look, but they show up easily using a dissecting microscope through the translucent cysts on the fins of infested fishes. I found success in the field using a handheld micrometer, probably similar to the one used by Rafael Araujo and his colleagues in Europe.

Westralunio carteri, like other Australian hyriids, appears to be a host generalist, utilising at least five and probably another two to three species of fishes to complete the larval to juvenile metamorphic stage of the mussel. The various species of host fishes might be important in the dispersal of *W. carteri*, as is suggested for other Unionoida in Strayer (2008). For instance, the fast-swimming and well travelled Western Minnow may be very important for wide-reaching dispersal and hence, genetic exchange between populations of *W. carteri*, while localised, heavily infested hosts such as gobies might be important for maintaining local abundance. The success or failure of attached glochidia to become metamorphosed juveniles is unclear, but Goldfish are unlikely to support the life cycle of *W. carteri*. Although I did not find any glochidia on the recently introduced Pearl Cichlid, nor was this species confirmed as a host in the laboratory, future studies should include a larger sample size given that it occurs in areas with abundant *W. carteri*. The poecilids (Eastern Gambusia and One-spot Livebearer), known elsewhere as 'Top minnows' are probably able to support the life cycle of *W. carteri*, at least locally, although I wonder if glochidia metamorphosis is as abundant as it would be if the natural host Western Pygmy Perch had not been displaced by these introduced fishes.

All I can say about the juvenile stage of *W. carteri* with any level of certainty is that they are difficult to find in nature without really intensive targeted sampling and that they easily succumb to flatworm and protozoan predation in the laboratory. On the few occasions I did find them in the field, they were in shallow water less than 20 mm deep mixed in with coarse sand during March, although I did not do any thorough searching given time constraints.

From the growth studies, we can see that growth rates and ages-at-length are quite variable between populations of *W. carteri*. A striking example of this was the major difference in ages of the largest of mussels when comparing the highly eutrophic Birrega Drain with the spring fed Bennett Brook. The mussels from Birrega Drain grew much faster and were as much as 10 years younger than those in Bennett Brook that were of comparable size. The use of calcein as a growth marker was effective in showing that growth rings occur annually. This work demonstrated the importance of validating growth and age for each population being studied and future work should focus on more long-term studies to better understand growth in the smallest and largest size classes. Determination of recent recruitment success and the factors which control it will be extremely important in determining long-term population viabilities of this threatened bivalve.

7.3 Using citizen science in the research process

Although species recovery, action plans and policies are a good start in species conservation protection, the real action comes from on-ground. I strongly support the notion that educating the community and getting ‘eyes on the ground’ are

important in species recovery and protection. Hence, 'Mussel Watch WA', discussed below.

Rather than this thesis ending up in the mountain of archival data stored in university libraries, which is not always readily available, I reached out to the public to give knowledge back to the community...after all, the actions and behaviour of the community will ultimately affect the fate of the species. Through many phone calls and emails, things came together during an initial meeting with Julie Robert from SERCUL. I spoke with Julie about my ideas for how to best approach the big task ahead of me in documenting the current distribution of the species, determining its conservation status and helping to protect it. Through more talks and meetings between myself, Julie, SERCUL staff, my supervisors and others, we wrote and received a generous grant from the Lotterywest Foundation to produce a series of educational materials on freshwater fauna of south-western Australia.

Along with three short films, produced by Ashley Ramsay (ENVfusion Films, Inc.), incorporating Nyungar knowledge with science on the freshwater fishes, crayfishes and mussels, as well as two educational brochures and a field guide, recently reviewed in *Pacific Conservation Biology* (Blake 2012), we came up with a novel website (http://www.musselwatchwa.com) to educate the public about freshwater mussels and our research on *Westralunio carteri*. Along with information, we included a downloadable and online form for people to provide us with distributional information to build into the distribution database. The response was excellent and I hope to provide more information and keep the website going once the results from this thesis are published.

7.4 Identification of knowledge gaps and implications for further research

There is still much to learn about *W. carteri*. Factors controlling recruitment success, habitat preferences and juvenile survival of *W. carteri* remain largely unexplored. A determination of longevity, through a long-term study committed to mussel life span and a targeted population census could better inform us about the likelihood of species survival or recovery into the future. Other host fish species need to be identified from the field, particularly the introduced salmonids and percids, and confirmed as competent hosts in the laboratory. In the bigger picture, questions of taxonomy (as I eluded to in Chapter 2), biogeography, parasitism and disease, nutrient cycling and provision of ecosystem services in this and other Australasian freshwater mussels remain unresolved and may prove to be valuable areas to pursue in the near future.

Perhaps the most rewarding aspect of my work was to enhance the profile of what may seem to most like an extraordinarily unexceptional invertebrate. Gaining recognition for this less than charismatic animal is akin to the eponymous character in the Brothers Grimm 1812 tale of Rumpelstizchen who spins straw into gold. Recognition of the importance of freshwater mussels as valuable components of a healthy freshwater ecosystem has yet to be realised in Australia. Given the chance, anyone who has been in the river surveying these critters will agree with me that despite the immediate lack of lustre, at the end of the day, community volunteers have left with comments like, “that was actually fun....like searching for treasure.” I have gained a true appreciation for the life that lurks beneath the surface of Western Australia’s waters, most of which is found nowhere else in the world.

Borrowing from an upcoming review of the conservation of the Australasian Hyriidae, I will leave the reader with a quote from Keith Walker who states, “For Hyriidae, the challenge is clear and the consequences of inaction are no less so. Freshwater mussels are founding members of the Gondwana fauna; they outlived the dinosaurs, but how many species will survive the modern era?”

Photo: Julie Robert

‘An understanding of the natural world and what’s in it is a source of not only a great curiosity but great fulfilment.’
-David Attenborough-

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